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Health Advisory for Hexachloroethane

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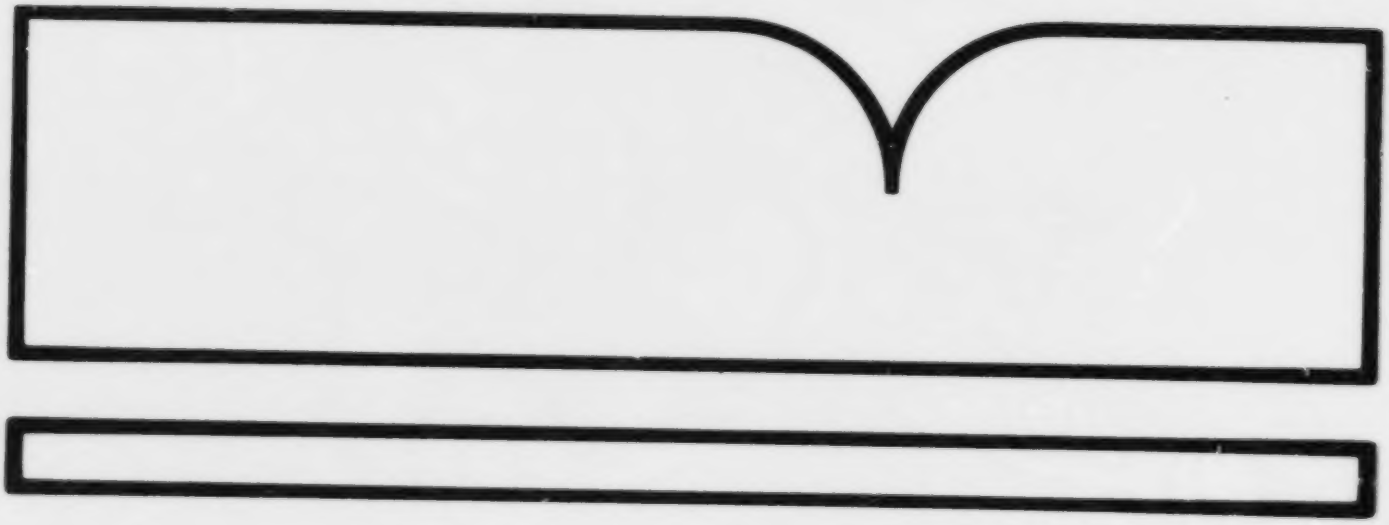

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HEXACHLOROETHANE

Health Advisory

Office of Drinking Water
U.S. Environmental Protection Agency
Washington, DC 20460

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16. ABSTRACT
This Health Advisory (HA) provides information on the health effects, analytical methodology and treatment technology that would be useful in dealing with Hexachloroethane contamination of drinking water. Based on available toxicity data the HA values for Hexachloroethane are: One-day, 5 mg/L; Ten-day, 5 mg/L; Longer-term (child), 0.1 mg/L; Longer-term (adult), 0.45 mg/L; and Lifetime, 0.001 mg/L. Hexachloroethane is classified as a possible human carcinogen. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. The HAs, developed by the US Environmental Protection Agency, Office of Drinking Water (ODW), are not legally enforceable Federal standards and are subject to change as new information becomes available. Health Advisories are developed for One-day, Ten-day, Longer-term and Lifetime exposures based on data describing noncarcinogenic end points of toxicity. For those substances that are known or probable human carcinogens, according to the EPA classification scheme, Lifetime HAs are not recommended. The chemical concentration values for carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value.

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Health Advisory on Hexachloroethane

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PREFACE

This report was prepared in accordance with the Memorandum of Understanding between the Department of the Army, Deputy for Environment Safety and Occupational Health (OASA(I&L)), and the U.S. Environmental Protection Agency (EPA), Office of Drinking Water (ODW), Criteria and Standards Division, for the purpose of developing drinking water Health Advisories (HAs) for selected environmental contaminants, as requested by the Army.

Health Advisories provide specific advice on the levels of contaminants in drinking water at which adverse health effects would not be anticipated and which include a margin of safety so as to protect the most sensitive members of the population at risk. A Health Advisory provides health effects guidelines, analytical methods and recommends treatment techniques on a case-by-case basis. These advisories are normally prepared for One-day, 10-day, Longer-term and Lifetime exposure periods where available toxicological data permit. These advisories do not condone the presence of contaminants in drinking water; nor are they legally enforceable standards. They are not issued as official regulations and they may or may not lead to the issuance of national standards or Maximum Contaminant Levels (MCLs).

This report is the product of the foregoing process. Available toxicological data, as provided by the Army, on the munitions chemical hexachloroethane (HCE) have been reviewed and relevant findings are presented in this report in a manner so as to allow for an evaluation of the data without continued reference to the primary documents. This report has been submitted to critical internal and external review by the EPA.

A companion document, "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for Hexachloroethane" is included in this report.

I would like to thank the authors, Ms. Loretta Gordon, Dr. William Hartley and Dr. Welford Roberts who provided the extensive technical skills required for the preparation of this report. I am grateful to the members of the EPA Tox-Review Panel who took time to review this report and to provide their invaluable input, and I would like to thank Dr. Edward Ohanian, Chief, Health Effects Branch, and Margaret Stasikowski, Director, Criteria and Standards Division, for providing me with the opportunity and encouragement to be a part of this project.

The preparation of this Advisory was funded in part by Interagency Agreement (IAG) 85-PP5869 between the U.S. EPA and the U.S. Army Medical Research and Development Command (USAMRDC). This IAG was conducted with the technical support of the U.S. Army Biomedical Research and Development Laboratory (USABRDL).

Krishan Khanna, Project Officer
Office of Drinking Water

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EXECUTIVE SUMMARY

Hexachloroethane (HCE), a chlorinated alkane, is a colorless to white crystalline material with a camphor-like odor. HCE can be dehalogenated by metals, alkalis, etc., to form spontaneously explosive chloroacetylenes. At 300 to 500°C, its vapor, in the presence of metal filings, decomposes to form phosgene and hydrogen chloride. Burning temperatures for the smoke mixtures range from 700 to 1,100°C and the products of pyrolysis are carbon and chlorine via carbon tetrachloride, tetrachloroethylene and hexachlorobenzene.

Hexachloroethane is manufactured by the chlorination of tetrachloroethylene in the presence of ferric chloride. Although the majority of HCE is imported, HCE is produced as a co-product in industrial chlorination processes.

Hexachloroethane has been used by the military in the production of pyrotechnic devices and screening smoke. The primary nonmilitary use of HCE has been as a precursor in the production of fluorocarbons which are used in dry-cleaning, aerosols and refrigerants. Other uses include pressure lubricants, rubber and insecticidal formulations, moth repellents, fire extinguishing fluids, fermentation retardant, chemical precursor, rodenticide, veterinary medicine and submarine paints.

The major transport mechanism of HCE from water and soil to the atmosphere is volatilization. Biotransformation is the major transformation process. Once in the atmosphere, HCE is thermodynamically stable. Adsorption to sediment and bioaccumulation of HCE may occur. Bioconcentration in aquatic species has been demonstrated.

Pharmacokinetics

Hexachloroethane can be absorbed upon inhalation, ingestion or dermal contact. It is preferentially accumulated in fat and the concentration in the kidney is higher in male rats than female rats. Several metabolites have been demonstrated both *in vivo* and *in vitro* and a reductive dechlorination has been proposed as the method of metabolism. Tetrachloroethene has been identified as the principle metabolite under anaerobic conditions with liver microsomal fractions. The main route of excretion appears to be expired air.

Health Effects in Humans

Data on the effects of HCE in humans is limited. HCE has been reported to adversely affect the central nervous system and the eye. The neurologic effects are mild, generally reported as an inability to close the eyelid, while direct effects on the eye include irritation, tearing, inflammation and photophobia.

Health Effects in Animals

Oral LD_{50}s in rats range between 5,160 and 7,690 mg/kg in males and 4,460 and 7,080 mg/kg in females. The oral LD_{50} in male guinea pigs was reported at 4,970 mg/kg. Toxic signs include tremor, ataxia, signs of gasping, and the appearance of a red exudate around the eyes.

An animal study of sheep indicated HCE produced liver toxicity at doses of 0.5, 0.75 and 1.0 g/kg. Kidney lesions, liver degeneration and necrosis and decreased body weight gain were exhibited in rabbits dosed at 320 and 1,000 mg/kg/day for 12 days. A significant decrease in body weight gain and development of gross liver and kidney lesions occurred in male rats when orally dosed with HCE at 200 and 500 mg/kg/day for 16 days. A No-Observable-Adverse-Effect-Level (NOAEL) of 50 mg/kg/day was determined based on this rat study.

A skin irritation study indicated that HCE is a mild skin irritant in rabbits at a dose of 0.5 g of the dry technical grade of HCE. An eye irritation study indicated that moderate corneal opacity, iritis, swelling and discharge occurred upon exposure of the cornea to direct contact with 0.1 g of the dry technical grade of HCE.

Exposure by inhalation at doses up to 200 ppm over a period of 6 weeks caused lesions of the nasal passage, trachea and lungs of rats and an increase in nasal mucous production in quail.

In a 16-week study, male rats administered HCE at doses up to 100 mg/kg/day developed kidney lesions. A NOAEL of 1.3 mg/kg/day was indicated based on this study.

No information is available on the effects of HCE on reproduction in animals. A study of developmental effects on rats administered HCE indicated that it was not teratogenic, although maternal and fetotoxic effects were observed, indicating a NOAEL of 100 mg/kg/day.

In a 78-week bioassay study for carcinogenicity with rats and mice, the mice developed hepatocellular carcinomas after exposure to HCE by oral intubation at doses of 590 and 1,179 mg/kg/day. The rats developed histopathological lesions that were limited to the kidney. However, there were no significant neoplastic lesions reported. No evidence of mutagenicity was detected in genotoxicity assays.

Quantification of Toxicological Effects

The 10-day Health Advisory (HA) is 5 mg/L for a 10 kg child based on the NOAEL of 50 mg/kg/day determined from the 16-day rat study. It is recommended that the 10-day HA be used as an estimate for the One-day HA because of lack of suitable data to calculate a One-day HA. The Longer-term HA is calculated to be 100 ug/L for the 10 kg child and 450 ug/L for the adult, based on a NOAEL of 1.3 mg/kg/day determined by the 16-week study of rats. The NOAEL of 1.3 mg/kg/day was also used to calculate a Reference Dose (RfD) of 1 ug/kg/day, a Drinking Water Equivalent Level (DWEL) of 40 ug/L and a Lifetime HA of 0.0007 mg/L or 1 ug/L.

Hexachloroethane is classified as a Group C carcinogen by EPA's guidelines, indicating it is a possible human carcinogen.

I. INTRODUCTION

The Health Advisory (HA) Program, sponsored by the Office of Drinking Water (ODW), provides information on the health effects, analytical methodology and treatment technology that would be useful in dealing with the contamination of drinking water. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. Health Advisories contain a margin of safety to protect sensitive members of the population.

Health Advisories serve as informal technical guidance to assist Federal, State and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. The HAs are subject to change as new information becomes available.

Health Advisories are developed for one-day, ten-day, longer-term (approximately 7 years, or 10 percent of an individual's lifetime) and lifetime exposures based on data describing noncarcinogenic end points of toxicity. For those substances that are known or probable human carcinogens, according to the Agency classification scheme (Group A or B), Lifetime HAs are not recommended. The chemical concentration values for Group A or B carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value together with assumptions for lifetime exposure and the consumption of drinking water. The cancer unit risk is usually derived from the linear multistage model with 95 percent upper confidence limits. This provides a low-dose estimate of cancer risk to humans that is considered unlikely to pose a carcinogenic risk in excess of the stated values. Excess cancer risk estimates may also be calculated using the one-hit, Weibull, logit or probit models. There is no current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than another. Because each model is based upon differing assumptions, the estimates that are derived can differ by several orders of magnitude.

II. GENERAL INFORMATION AND PROPERTIES

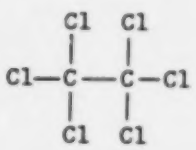
Hexachloroethane (HCE), a chlorinated alkane, is a colorless to white rhombic crystalline substance with a camphor-like odor detectable at 0.15 ppm. At higher temperatures, >46°C and >71°C, its crystalline form is triclinic and cubic, respectively (Santodonato, 1985). Since the 1930's, HCE has been used primarily by the military, in combination with zinc oxide and aluminum, in pyrotechnic devices and as a screening smoke. It has had more limited usage in extreme pressure lubricants, rubber and insecticidal formulations, moth repellents and fire extinguishing fluids, and has also been used as a fermentation retardant, a chemical precursor and rodenticide. The antihelminthic properties of HCE have resulted in its use in veterinary medicine. It has also been included as a component of submarine paints, paper, wood and related products (Sax, 1986).

While nonflammable, HCE can be dehalogenated by metals, alkalis, etc. to form spontaneously explosive chloroacetylenes (Sax, 1986). At 300 to 500°C, its vapor, in the presence of metal filings, decomposes to form phosgene and hydrogen chloride (≈1:8 at 450°C in the presence of iron) (Sjoberg, 1952 as cited in Dacre et al., 1979). Burning temperatures for the smoke mixtures range from 700 to 1,100°C and the products of pyrolysis are reported to be carbon and chlorine via carbon tetrachloride, tetrachloroethylene and hexachlorobenzene (Jarvis, 1970 as cited in Dacre et al., 1979). The general chemical and physical properties of HCE are presented in Table II-1.

First produced commercially in the United States in 1921, HCE is manufactured by the chlorination of tetrachloroethylene (perchloroethylene)in the presence of ferric chloride at temperatures ranging between 100 to 140°C. When the process is 50 to 60% complete, the reaction is stopped, neutralized with alkali and the HCE is crystallized and separated via centrifugation (Kirk-Othmer, 1964 as cited in NIOSH, 1981). Under pressure and at lower temperatures, <60°C, HCE may also be produced by the photochemical chlorination of the tetrachlorinated ethylenes (Archer, 1979 as cited in Davidson et al., 1988). Smaller quantities of the highly purified compound have been produced by the action of chlorine on barium chloride (Jondorf et al., 1957). Current production in the U.S. is generally limited to its formation as a co-product in the manufacture of other chlorinated ethanes, followed by its recycling as feedstock or its thermal oxidation (Archer, 1979 as cited in Santodonato, 1985). The U.S. Department of Commerce reported an average annual import of approximately 1.6 million pounds of HCE from several European sources between 1973 and 1979 (NIOSH 1981).

Smoke compositions containing HCE, at levels currently ranging from 43.5 to 46.5%, include smoke pots and canisters, grenades, cartridges and projectiles. The HCE, used as a chlorine carrier, is premixed with zinc oxide with aluminum added and blended prior to loading (Davidson et al., 1988). Laboratory analysis of the by-product gases from HCE-containing smoke pots indicate that perchloroethylene (tetrachloroethylene) is the major component (3 to 17% of reagent weight) while carbon tetrachloride (1–3%), hexachloroethane (0.3–5%),

TABLE II-1 General Chemical and Physical Properties of Hexachloroethane

CAS Number	67-72-1
Synonyms	carbon hexachloride, perchloroethane, ethanehexachloride, 1,1,1,2,2,2-hexachloroethane, hexachlorethylene, avlothane, distokal, distopan, distopin, egitol, falkitol, fasciolin, hexoram, phenohep
Molecular Weight	236.74
Chemical Formula	C_2Cl_6
Structure	
Physical State	white solid, rhombic crystal
Boiling Point	186.8°C (triple point)
Melting Point	186.8–187.4°C (sealed tube) (sublimes)
Density	2.091 (20°C)
Vapor Pressure	0.4 mm Hg (20°C)
Solubility	50 mg/L in water (22°C), very soluble in alcohol, ether; soluble in benzene, chloroform and oils
Octanol/water partition coefficient Log K_{ow}	3.34 (measured)
Odor Threshold	0.15 ppm
Conversion Factor (air)	1 ppm = 9.68 mg/m^3 (25°C; 760 mm Hg)

References: IARC, 1979; Amoore and Hautala, 1983 as cited in Santodonato, 1985; Sax, 1986; Verschueren, 1983; Weast, 1986; Windholz, 1983

phosgene (0.1–1%) and hexachlorobenzene (0.4–0.9%) are the other major gases produced. A burn efficiency of 70% is assumed (Katz et al., 1980). Zinc chloride is the major metallic by-product of these tests with a reagent weight mass of approximately 48% (Novak et al., 1987). Average annual use of HCE in the manufacture of smoke devices was reported to be approximately 193,000 lbs in the period between 1966 and 1977. If smoke device production was increased to full capacity (e.g., to support a full military mobilization), the use of HCE could increase to a rate of approximately 750,000 lbs/month (Kitchens et al., 1978)

III. OCCURRENCE

Hexachloroethane is not known to occur in nature (IARC, 1979), however, its introduction into the environment can arise from its military and industrial uses and during its manufacturing processes. It has been heavily used by the military since World War II in the production of various smoke and pyrotechnic devices. Its primary nonmilitary use had been as a precursor in the production of fluorocarbons which are used in drycleaning, aerosols, refrigerants and similar products (Kitchens et al., 1978).

While the majority of the HCE currently used in U. S. industries is imported, with the last report of U.S. manufacture being in 1967 (IARC, 1979), it is produced as a co-product in industrial chlorination processes and is utilized in the manufacture of C_2-chlorinated hydrocarbons (Archer, 1979 as cited in Santodonato, 1985). In 1987, DeMarini et al. indicated that the U.S. EPA (1984) had measured HCE at a level of approximately 560 ug/g of hazardous waste sample from a petrochemical manufacturing plant.

In 1978, Kitchens et al. reported that the production rate for HCE containing devices at Pine Bluff Arsenal, the major facility for the production of pyrotechnic devices in the U.S., required the use of approximately 15,000 lbs of HCE/month. This use rate represents approximately 2% of the production rate possible under full mobilization capacity. Losses of the HCE during the production process, which includes mixing, transfer, pressing and sealing of the canisters, has been estimated at 1 to 2% of the total chemical used or approximately 150–300 lbs/month. Washdown of the assembly areas also contributes to this loss but is relatively minor. Up to 1979, the Pine Bluff facility produced approximately 117,000 gallons of wastewater/8 hr day from 23 buildings in the pyrotechnic area of the facility. The estimated average concentration of HCE in the wastewater during this time was 168 mg/L with most of the wastes eventually discharged into the Arkansas River in which the substantial flow rate provided a large volume of dilution water. The installation of a new pollution abatement facility, placed online in 1979, resulted in no HCE being detected in wastewater samples from five areas of the facility (Fortner et al., 1983 as cited in Davidson et al., 1988). Burning of off-specification formulations and collected solid wastes was also a source of entry of HCE into the environment. Installation of a new incineration facility is anticipated to reduce this source of pollution (Kitchens et al., 1978).

Its use by the military in chemical obscurant and screening smoke compositions is another potential major source of HCE in the atmosphere with its subsequent entry into the water supply via atmospheric fallout, runoff and soil or sediment leaching. Although the production of HCE-containing smoke devices is limited to the Arkansas facility, it is widely used by numerous training bases both within and outside the U.S. While HCE represents approximately 44.5% of the chemical composition of smoke pots and similar devices, analysis of its combustion products during smoke generation indicates that the major product of such combustion is zinc chloride ($ZnCl_2$). Hexachloroethane represents approximately 0.3 to 5% of the gaseous products of this combustion. Shinn et al. (1985) estimated maximum environmental concentrations of smoke

from the various HCE devices following detonation and determined a range of 10–110 mg/m^3 for diffusing devices such as the smoke pots, and a range of 220–660 mg/m^3 for grenade and howitzer diffusing devices. Concentrations of HCE vapor, at a distance of approximately 15 cm from the mouth of a typical HCE smoke pot device, was measured at approximately 40 ppm (≈ 385 mg/m^3; Katz et al., 1980).

The area most significantly impacted during the detonation of such devices is generally local, usually limited to isolated base areas 5,000–15,000 m (3.1 to 9.3 miles) from populated areas, with vapor carrying to those areas approximately 10–15 km (6.2–9.3 miles) downwind (Novak et al., 1987). The minimum area impacted for HCE diffusing devices was estimated by Shinn et al. (1985) to have a range of 50–1,200 m^2 (slightly more than one quarter of an acre, maximum) for howitzers and grenades and a range of 9,500–65,000 m^2 (approximately 16 acres, maximum) for smoke pots.

In the U.S., HCE has been detected in the effluent from a chemical plant, a chlorinated sewage treatment plant (Shackelford and Keith, 1976) and a kraft paper mill (Keith, 1976). It also has been found in river water and in surface water from only 1 of 204 sites near heavy industrial areas (Ewing et al., 1977). HCE was detected in drinking water from 4 of 13 cities sampled (Keith et al., 1976) and in eight samples of finished drinking water (Shackelford and Keith, 1976 as cited in IARC, 1979). It has also been detected in soil from the Love Canal chemical dump site and in the troposphere (Davidson et al., 1988).

In an ongoing EPA study as well as other studies, HCE has been measured in manufacturing effluent and wastewaters from industrial and publicly-owned treatment facilities at levels ranging from 0.9–1,405.6 ug/L (Ewing et al., 1977; Keith, 1976 as cited in Davidson et al., 1988). In 1982, Oliver and Nicol (as cited in Oliver and Niimi, 1983) reported that environmental concentrations of HCE in Lake Ontario were measured in the range of 0.02 ng/L while Oliver and Kaiser (1986 as cited in Davidson et al., 1988) reported levels of 0.02 to 1,700 ng/L in St. Clair River water and from 1.4 to 530 ng/L in suspended sediment. Levels between 1–3 ug/L have been measured in two surface water samples from 204 sites near 14 heavily industrial river basins in the U.S. (Ewing et al., 1977 as cited in Davidson et al., 1988).

Contamination of ground water with HCE has been reported to have occurred from leachate arising from a pesticide manufacturing waste dump in Hardeman County, Tennessee. The U.S. EPA (1979) measured HCE in 19 of 31 samples taken from private wells in the contaminated area with levels ranging from trace to 4.6 ug/L and a median level of 0.26 ug/L (Clark et al., 1982). Keith et al. (1976 as cited in IARC, 1979) reported levels of 0.03 to 4.3 ug/L in drinking water in 4 of 13 cities while Suffet and Radziul (1976 as cited in Davidson et al., 1988) reported the presence of HCE in the Torresdale drinking water supply for the city of Philadelphia which comes from the tidal waters of the Delaware River.

In 1984, Silkworth et al. (as cited in Davidson et al., 1988) reported a level of 1.0 ug/g in the soil taken from the dump site at the Love Canal and

0.3–49 ug/m^3 in air samples from a cage containing the HCE-contaminated soil. Finally, HCE has been measured in the troposphere of the northern hemisphere at 0.17 parts per trillion (ppt) by volume and in the southern hemisphere at 0.25 ppt by volume. The primary source of this contamination has been reported to be from the production of chlorinated C_2 hydrocarbons (Singh et al., 1980; Class and Ballschmitter, 1986 as cited in Davidson et al., 1988).

IV. ENVIRONMENTAL FATE

Relatively little data are available on the environmental fate of HCE in water. Experimental data indicate that volatilization is an important process in the transport of HCE from water and soil to the atmosphere and that biotrans-formation will also occur. Once transported to the atmosphere, it is predicted that HCE will be stable. Adsorption to sediment and bioaccumulation have also been demonstrated.

Callahan et al. (1979) reported on two experiments conducted by Dilling et al. (1975) and Dilling (1977) in which an experimental half-life for volatilization of HCE from water was reported to be 45 minutes, based on a concentration of 1 mg/L in an open, 250 mL container. Evaporation rate was directly proportional to the rate of stirring. In a continuation of this study, an evaporative half-life of 40.7 minutes for 0.72 mg/L in stirred water was reported. This second experimental result corresponded well with the theoretical equation of MacKay and Leinonen (1975) as cited in Callahan et al. (1979) which predicted a half-life of 38 minutes. In 1985, Spanggord et al. calculated a volatilization rate constant for HCE of 0.01 hr^{-1} for a body of water 180 cm deep with an estimated half-life of 70 hours. The Henry's Law constant of 6,100 L-torr $mole^{-1}$ indicates a rapid volatilization rate in shallow, turbulent water.

In a series of experiments designed to determine if biotransformation of HCE occurs in water, under either aerobic and anaerobic conditions, Spanggord et al. (1985) determined that, in an open container of aerated pond water, sterile and non-sterile HCE rapidly disappeared, indicating that volatilization may be a competitive process. When sediment was present in the pond water, an additional peak developed after 6 days of incubation, indicating that biotransformation was probably occurring. Under anaerobic conditions, the pond water, either with sediment or with the addition of glucose plus yeast extract, lost 90% of its HCE content in 18 days while the HCE content of sterile pond water remained unchanged.

When organisms from the pond water sample were transferred to incubation media, the formation of metabolites was observed, along with a decrease in the HCE concentration under both aerobic and anaerobic conditions. The authors concluded that biotransformation is a viable process for HCE and that the process is more rapid under anaerobic than under aerobic conditions, where volatilization is the predominating factor. Spanggord et al. (1985) also confirmed that HCE is biotransformed in soil and suggested that biotransformation is the major transformation process, while volatilization is the major transport process of HCE from soil and water.

In 1986, Criddle et al. studied the fate of HCE in an unconfined sand aquifer and determined a half-life of approximately 40 days, with aerobic conditions prevailing. Additional laboratory studies indicted that the HCE was reductively biotransformed to tetrachloroethylene in aerobic cultures containing either municipal wastewater microflora or aquifer microbial materials. In the wastewater study, the transformation was reported to be constant with time. In the aquifer study, the combined concentrations of HCE and tetrachloro-

ethylene were less than the amounts added at the start of the study. The combined concentrations decreased with time which indicated the possibility of a slow increase in sorption with time. The conversion of HCE to PCE was not uniform in all aquifer samples, suggesting uneven distribution of the active agents, and autoclaving of the sample did not prevent transformation although it appeared to enhance sorption of the HCE.

Bioconcentration of HCE in aquatic species has been confirmed. In 1982, Barrows et al. determined a bioconcentration factor (BCF) of 139 for HCE in bluegill sunfish after an exposure for 28 days in water with a mean HCE concentration of 6.17 ug/L. The BCF is defined as the quotient of the mean measured residues of a compound in whole body fish tissue during the equilibrium period divided by the mean measured concentration of the compound in exposure water. Bioconcentration factors greater than unity indicate that accumulation of the chemical has occurred in the tissue. When the exposed fish were transferred to an aquarium containing fresh water, a half-life of <1 day was determined, indicating that biological persistence is low.

Bioconcentration of HCE and other chlorinated hydrocarbons from water at environmental concentrations (ng/L range) was demonstrated by Oliver and Niimi (1983) in rainbow trout. The study was designed to determine if the BCF was dependent on the concentration of the chemical in water, particularly when exposure was at ambient or only slightly higher than environmental levels. Additionally, the BCF at the higher test level averaged 2.4 times that at the lower level. In this study, control water contained approximately 0.03 ng/L of HCE, low level test water, 0.32 ng/L and high level exposure water, 7.1 ng/L. The BCF was 510 and 1,200 for the low and high exposure tests, respectively. Furthermore, the experimental BCF developed in this study was used to predict the concentration of HCE in fish in Lake Ontario water where the concentration of HCE had been measured at 0.02 ng/L (Oliver and Nicol, 1982 as cited in Oliver and Niimi, 1983). The predicted value of 0.01 ng/g for HCE correlated well with the mean concentration of 0.03 ng/g of HCE in Lake Ontario fish.

Spanggord et al. (1985) also determined a relatively low soil sorption partition coefficient and suggested that HCE should migrate fairly rapidly in soil. No chemical transformation processes that dominate in soil, water or air were identified in this study. Curtis et al. (1986) as cited in Davidson et al. (1988) reported an uptake for HCE in laboratory studies that was characterized by an initial rapid sorption step (<2 hrs) that accounted for approximately 50% of the total sorption, followed by a gradually decreasing rate. An equilibration time of 3 days was determined and the sorption and desorption isotherms were reported to be linear and reversible at concentrations ranging between 1–50 ug/L. The authors indicated that partitioning of HCE into organic matter was the observed mechanism for the sorption process.

The photooxidation of HCE by ultraviolet light in the presence of water was studied by Knoevenagel and Himmelreich (1976) as cited in Davidson et al. (1988). A half-life of 93.7 hours for 237 mg of HCE in 900 mL of water at a

temperature of 90 to 95°C under aerobic conditions was determined. The photodegradation was measured by the amount of carbon dioxide formed. Under anaerobic conditions, the cleavage occurs at the C-Cl bond.y

When released to the atmosphere by volatilization, HCE is thermodynamically stable, no degradation by hydroxyl radicals is expected, and the tropospheric lifetime is expected to be quite long (Davidson et al., 1988). A total transport within the atmosphere and between the atmosphere and oceans of approximately 1 kton/yr was predicted by Class and Ballschmitter (1986 as cited in Davidson et al., 1988).

V. PHARMACOKINETICS

Hexachloroethane, which can be absorbed following inhalation, ingestion or dermal contact, is preferentially accumulated in fat. In male rats, kidney levels are significantly increased over that of females. Several metabolites have been demonstrated both *in vivo* and *in vitro*, and a reductive dechlorination has been proposed as the method of metabolism. Tetrachloroethene has been identified as the principle metabolite under anaerobic conditions with liver microsomal fractions. The main route of excretion appears to be expired air with urinary excretion playing a relatively minor role.

A. Absorption

Absorption of HCE by ingestion was demonstrated by Fowler (1969) in Scottish Blackface male sheep administered, by drenching bottle, a dose of 0.5 g/kg of HCE in olive oil emulsified with water. Absorption was slow with HCE first detected in the blood of an anesthetized male after 27 minutes post-dosing. Hexachloroethane was detected in the bile 15 minutes after dosing. Total recovery (percent of dose) was not determined in this study. In a study by Jondorf et al. (1957) in rabbits fed HCE at a level of 0.5 g/kg in the diet, only 19 to 29% of the administered radioactivity was accounted for in 3 days with 5% recovered in the urine and the remainder in expired air.

The maximum tolerated dose (MTD), as determined in the NCI (1978) bioassay of HCE, was administered to male Osborne-Mendel rats and male B6C3F1 mice. They were dosed by intubation 5 days/week for 4 weeks, followed by a single dose of radiolabelled HCE. Between 93–95% of the administered radioactivity was recovered over 48 hours (Mitoma et al., 1985). Doses in this study were 500 mg/kg/day (2.11 mmol/kg) for rats and 1,000 mg/kg/day (4.22 mmol/kg) for mice. Table V-1 compares the recovery in both species. In contrast to the Fowler (1969) study in sheep, between 65–72% of the radioactivity administered to rats and mice was recovered in expired air while only 6–16% was recovered in the combined excreta.

B. Distribution

Studies on the distribution of HCE have been reported in two male Scottish Blackface sheep following an oral dose of 0.5 g/kg (Fowler, 1969) and in male and female CDF Fisher 344 rats (4/dose) after 16 weeks on test diets containing HCE at levels of 1, 15 or 62 mg/kg/day (Gorzinski et al., 1985). In both studies, the majority of the HCE was recovered from the fat. Table V-2 compares the tissue levels, in ug HCE/g tissue, in rats receiving the high dose of 62 mg/kg/day for 16 weeks and in sheep at 8.5 hours after a single dose of 500 mg/kg.

In the rat study, the concentration of HCE in the kidneys of the male rat was higher than in females and was proportional to dose (Table V-3). Tissue levels in the kidneys of females receiving the low dose amounted to approximately 27% of the level detected in the males while the levels in

Table V-1 Percentage of ^{14}C-HCE Recovered in 48 Hours in Rats and Mice Following Multiple Doses[a]

	Rat (500 mg/kg/day)[b]	Mouse (1,000 mg/kg/day)[c]
Total Recovery	93.3	95.5
Expired Air	64.6	71.5
CO_2	2.4	1.8
Excreta	6.3	16.2
Carcass	20.0	5.9

Reference: Mitoma et al. (1985)

[a] Standard deviations omitted.
[b] 2.11 mmol/kg
[c] 4.22 mmol/kg

Table V-2 Concentration of HCE in Tissues Following Single (Sheep) or Multiple (Rats) Oral Doses[a]

	Rats (62 mg/kg/day)		Sheep (500 mg/kg)[b]
	Male	Female	Male
Liver	0.71	0.63	0.2
Kidney	95.12	2.01	0.1
Fat	176.1	162.1	1.7
Brain	--(c)	--	0.2
Muscle	--	--	0.04
Blood	0.74	0.61	0.2
References:	Gorzinski et al. (1985)		Fowler (1969)

[a] Concentration in ug HCE/g tissue for 4 rats/sex and 1 sheep
[b] anesthetized for bile studies
[c] not analyzed

females at the two higher doses were only about 2–3% of the levels in males. The increase of HCE content of the fat in both species was proportional to dose. A disproportionate increase in HCE content of the liver and blood was evident in both species. Table V-3 compares the tissue-to-blood concentration ratios for both sexes. When compared in this manner, a dose-related increase of the HCE in the kidneys of male rats is clearly evident while that in the female kidney appears to decrease slightly with increasing dose. Concentration ratios for liver in both sexes are remarkably similar. In contrast, the concentration ratio for HCE in the fat of the female rats, while similar to males at the low and high dose, is >4 times higher than males at the mid-dose level. This phenomena may be due, at least in part, to the low concentration of HCE in the blood of females at this dose level.

Gorzinski et al. (1985) treated an additional group of male CDF Fischer-344 rats with the high dose of HCE (62 mg/kg/day) in the diet for 8 weeks followed by 3, 6, 13, 22 or 31 days on control feed to obtain data on tissue clearance rates. Samples of fat, liver, kidney and whole blood were analyzed for HCE content. The concentrations of the HCE were found to decrease in the tissues in an apparent first-order manner, with tissue half-lives between two and three days. With an average half-life of 2.5 days, the level of HCE in the rat was calculated to have reached a 99% steady state concentration in 17 days. The authors indicated that the data did not seem to suggest accumulation in the tissue with increasing duration of treatment.

C. Excretion

Jondorf et al., (1957) reported that rabbits excreted 5% of the administered radioactivity in the urine in three days following the feeding of 0.5 g/kg of ^{14}C-HCE in the diet. Between 14–24% of the radioactivity was recovered in the expired air during the same time period with the remainder unaccounted for.

In contrast, Mitoma et al. (1985) indicated that Osborne-Mendel rats and B6C3F$_1$ mice excreted 64.6 and 71.5%, respectively, of the administered radioactivity in expired air following oral dosing with ^{14}C-HCE 5 days/week for 4 weeks. Recovery in the combined excreta was 6.3 and 16.2% for rats and mice, respectively, 48 hours after the last dose. Doses for this study, the MTDs, were 500 mg/kg/day for rats and 1,000 mg/kg/day for mice.

Fowler (1969) measured HCE recovery from urine and feces in two sheep that were given a single oral dose, 0.5 g/kg, of the chemical. More than 80% of the total HCE measured in the feces, an average of approximately 1,000 ug, was recovered within the first 24 hours. Recovery from the urine was approximately 6% of that found in the feces and was also largely excreted in the first 24 hours. Recovery from expired air was not reported.

Table V-3 Tissue-to-Blood Concentration Ratio[a] after Oral Administration of HCE to Rats

Dose mg/kg/day[b]	Liver		Kidney		Fat	
	Male	Female	Male	Female	Male	Female
1.0 (0.079/0.067)	3.7	3.9	17.2	5.5	39.9	38.6
15 (0.596/0.162)	2.9	2.9	40.8	4.2	63.6	279.4
62 (0.742/0.613)	1.0	1.0	128.2	3.3	237.3	264.4

Adapted from Gorzinski et al. (1985)

[a] ug/g tissue per ug/mL blood.
[b] The numbers in parentheses are the concentrations of HCE (in ug/mL) in the blood of male/female rats, respectively.

D. Metabolism

Fowler (1969) reported the occurrence of two metabolites of HCE in the hexane extracts of both the urine and feces of sheep following its oral administration at a dose of 0.5 g/kg. Tetrachloroethylene (perchloroethylene) was the major metabolite, present in amounts approximately equal to unmetabolized HCE in the feces (≈1,050 ug) but urinary recovery was roughly 50% that of HCE in the feces. The second metabolite, pentachloroethane, was present at levels from trace to approximately 450 ug in the feces and at levels similar to TCE (≈25 ug) in the urine. Analysis was by gas-liquid chromatography.

Trichloroethanol and trichloroacetic acid were reported as the major metabolites in rats and mice following the oral administration of ^{14}C-HCE at the MTD of 500 and 1,000 mg/kg/day, respectively, for 5 days/week over 4 weeks (Mitoma et al., 1985). Urinary metabolites, extracted with methanol, were analyzed by high performance liquid chromatography (HPLC) coupled to a radioactive flow detector. The authors reported that between 24 and 29% of the HCE was metabolized, as measured by the combined recovery in the excreta, carcasses and as CO_2.

Jondorf et al. (1957) reported the recovery of six metabolites in the urine of rabbits following the oral administration of 0.5 g/kg of ^{14}C-HCE in the diet. The metabolites, ranging from 0.1 to 1.3% in the urine were identified by chromatographic and isotope-dilution techniques as trichloroethanol, dichloroethanol, trichloroacetic acid, dichloroacetic acid, monochloroacetic acid and oxalic acid. In expired air, recovered metabolites were identified as carbon dioxide, tetrachloroethylene, and 1,1,2,2-tetrachloroethane along with unmetabolized HCE.

In studies conducted under anaerobic conditions using the liver microsomes from phenobarbitone-pretreated rats, HCE was reductively dehalogenated by NADPH. The process was found to be dependent upon the presence of cytochrome P450 (Nastainczyk et al., 1982). The authors further postulated that the reaction proceeds via a two-electron reduction mechanism producing first a radical and then a carbanion. The metabolites were identified as tetrachloroethene (99.5%) and pentachloroethane (0.5%). Additional metabolites seen in in vivo systems may arise from further reduction processes.

Town and Leibman (1984) further described the conditions for the reductive dehalogenation process. The reaction occurs, for the most part, in the microsomes; it is inhibited in the presence of oxygen, and the optimum pH is 7.0–7.5. Inhibition of cytochrome P450 resulted in an inhibition in the formation of the tetrachloroethylene. No tetrachloroethylene could be detected when rabbit liver microsomes were used. In a study by Salmon et al. (1985), dechlorination of halocarbons using a vesicular reconstituted system of cytochrome P450 enzymes from rabbit liver was similar to that catalyzed by rat liver microsomes, indicating that the system requires both reductase and a phenobarbital inducible form of cytochrome P450.

VI. HEALTH EFFECTS

Few data are available on the effects of HCE in humans. Laboratory studies in animals have identified the kidney as the principle site of toxicity. No data are available on the reproductive effects of HCE, but it does not appear to be teratogenic nor genotoxic. Evidence is presented on the carcinogenic effects of HCE in mice.

A. Health Effects in Humans

In 1978, the National Institute for Occupational Safety and Health (NIOSH), in a National Occupational Hazards Survey conducted from 1972–1974, estimated that 1,500 workers were potentially exposed to HCE in the workplace (Parker et al., 1979). As is the situation with most chloroethanes, HCE has been reported to adversely affect both the central nervous system (CNS) and the eye. Its neurologic effects are mild, generally reported as an inability to close the eyelid. Its direct effects on the eye include irritation, tearing, inflammation and photophobia upon exposure to fumes from heated HCE (Grant, 1986).

No data on the acute or chronic toxic effects of HCE exposure in humans were found in the English language literature. Plotnikov reportedly ingested 30 g of HCE in three days while Sokolov ingested 48 g in 4 days, with reduced skin sensitivity the only reported effect (Plotnikov and Sokolov, 1947, cited in Dacre et al., 1979). Saric and Knezevic (1957 as cited in Dacre et al., 1979) reported irritation as the most frequent complaint among industrially exposed workers. A change in the serum albumin:globulin (AG) ratio was the only clinical finding.

A report of death and significant injury due to inhalation of fumes from an HCE smoke grenade (Fischer, 1969 as cited in Dacre et al., 1979) was attributed to the presence of zinc chloride ($ZnCl_2$), a major ingredient of the smoke product.

B. Health Effects in Animals

1. Short-term Exposure

Early studies in sheep indicated that HCE produced some degree of liver toxicity, but similar findings were not seen in rats following a single oral dose. When male rabbits were similarly treated for 12 days, both liver and kidney toxicity were seen at the two high dose levels. Exposure of male and female rats for 16 days to HCE in the diet resulted in kidney and liver toxicity in the males. Exposure by inhalation caused lesions of the nasal passage, trachea and lungs of rats and an increase in nasal mucous production in quail, but no similar effects in dogs or guinea pigs.

Initial reports on the oral toxicity of HCE to animals were associated with the use of HCE in the treatment of mature liver flukes (Fasciola hepatica) in sheep and cattle. Olsen (1947) as cited in Fowler (1969) reported that

adverse reactions among the treated sheep included intoxication, incoordination, muscle tremors and death. Using castrated and uncastrated male Scottish Blackface or Cheviot cross sheep (15–23 kg), Fowler (1969) studied the effects of HCE. The HCE was dissolved in olive oil (15% w/v) and emulsified with water in the presence of gum acacia (12.5% w/v) and gum tragacanth (0.7% w/v). The solution was orally administered by drenching bottle at doses of 0.5, 0.75 or 1.0 g/kg (10, 1 and 1 animals/dose level, respectively). Hepatic toxicity was evaluated by measuring plasma levels of glutamate dehydrogenase (GD), sorbitol dehydrogenase (SD), ornithine carbamoyl transferase (OCT) and aspartate aminotransferase (GOT). A bromsulphthalein (BSP) dye clearance test also was conducted to assess liver function.

The doses were well tolerated with slight tremor of the facial muscles reported in three of the animals within the first 4 hours after dosing but with no effect on their ability to eat. Administration of HCE resulted in a simultaneous increase in all of the plasma enzymes, with maximum levels evident at 48 hours after dosing and a return to normal levels by 4 to 5 days post-dosing. Increases averaged from three to six times normal levels for GD and SD and two to ten times for OCT. The GOT levels increased slightly. The BSP clearance test indicated the HCE had no affect on the uptake from plasma to liver cells, but a marked reduction in the transfer from liver to bile was evident at 72 hours after dosing with HCE. This study indicated that HCE, orally administered to sheep, resulted in a degree of hepatic dysfunction. The elevated plasma enzymes suggested to the authors that HCE caused cell membrane permeability changes, probably at the intracellular level, while the decreased BSP dye clearance and unchanged hepatic uptake rate indicated a decrease in the excretory capacity of the liver.

In a study conducted by Weeks and Thomasino (1976), six male Sprague-Dawley, Wistar strain rats per level were administered a single oral dose of a 50% solution of HCE in corn oil with doses ranging from 2,510 to 10,000 mg/kg. Animals were observed for the occurrence of toxic signs, mortality was recorded, and the survivors were observed for 14 days after dosing. A gross pathological examination was performed on all dosed animals.

The acute oral LD_{50} level (95% confidence limits) in the male rats was 5,160 (4,250–6,270) mg/kg. Toxic signs were evident within 4–12 hours after dosing and included the appearance, at all dosage levels, of a red exudate around the eyes which persisted throughout the 14-day observation period. At doses of 5,010 mg/kg and higher, the animals developed tremors, ataxia and signs of gasping. Mortality usually occurred within the first 12–24 hours to 4 days after dosing with one delayed death on Day 7. No gross lesions were observed in any of the surviving rats while one animal that died after dosing showed evidence of degeneration of the cortex of the kidney. The acute oral LD_{50} for female rats, calculated by the method of Finney (1971), was reported to be 4,460 (3,900–5,110) mg/kg (Weeks et al., 1979). An approximate lethal dose (ALD - first dose of the lowest series of three ascending doses, all of which produced fatalities) of 4,900 mg/kg was determined after oral administration of HCE to male rats (Weeks and Thomasino, 1976) and of >1,000 mg/kg in male rabbits (Weeks et al., 1979).

Weeks et al. (1979) also reported the acute oral LD_{50} for HCE, dissolved in methylcellulose (5% w/v), in male and female Sprague-Dawley rats to be 7,690 (6,390–9,250) and 7,080 (6,240–8,040) mg/kg, respectively. An oral LD_{50} of 4,970 (4,030–6,150) mg/kg was reported in male Hartley guinea pigs for HCE dissolved in corn oil (50% w/v). Calculations were by the method of Finney (1971).

The effects of HCE on the liver were studied in four male Charles River rats (150–300 g) fasted for 16 hours prior to the administration by gavage of a single dose of HCE in mineral oil at a level of 2,600 umoles/100 g animal (equivalent to ≈ 6 g/kg) (Reynolds, 1972). No significant effects were seen on the chemical and functional properties of the liver microsomes as compared to the controls and measured at 1 to 2 hours after dosing.

The development of toxic signs and changes in blood chemistry parameters were studied in male New Zealand White rabbits (5/group) following the oral administration of HCE (99.8% pure) suspended in 5% aqueous methylcellulose at doses of 0, 100, 320 or 1,000 mg/kg/day for 12 days (Weeks et al., 1979). Body weights were recorded daily and blood samples, drawn from the central ear artery, were taken on Days 1, 4, 8, 12 and 4 days after termination of treatment. Serum levels for glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), alkaline phosphatase, bilirubin, total protein, potassium and sodium were determined. The animals were sacrificed on Day 4 after the last dose administration, major organs were weighed and samples of tissues were prepared for microscopic examination.

At the high dose level, toxicity was evidenced by a significant reduction in body weight, beginning at Day 7, with a significant increase in the relative liver and kidney weight ratios at termination. At the 320 mg/kg/day dose level, body weight reduction became significant by Day 10 of dosing. No changes in body weight were seen in the low dose group (100 mg/kg/day) and no other toxic signs were evident.

Administration of HCE at the two higher doses resulted in a significant decrease in the serum potassium and glucose levels. A trend toward increasing serum alkaline phosphatase, SGOT and bilirubin levels was seen in the high dosed group but no statistical significance was evident. No other blood chemistry parameters were affected.

Upon necropsy, signs of liver degeneration and necrosis were seen in animals receiving the two highest dose levels. Histopathological examination revealed dose related changes in the liver to include fatty degeneration, coagulation necrosis, hemorrhage and ballooning necrosis. Eosinophilic changes and hemosiderin-laden macrophages and giant cells were also evident. No signs of liver toxicity were seen at the lowest dose level.

Toxic tubular nephrosis of the convoluted tubules of the corticomedullary region of the kidney were seen in the two high dosed groups but the response was not considered to be dose related. Minimal tubular nephrocalcinosis was also seen at these two levels. No toxic effects were observed in the kidneys

of the low-dosed group. Based on the data from this study, a dose of 100 mg/kg/day can be considered the No-Observed-Adverse-Effect-Level (NOAEL) for the effects of HCE on the kidneys of male rabbits.

In a 16-day tolerance study, Gorzinski et al. (1980a) fed male and female CDF Fisher 344 rats (number per group not specified) a diet containing HCE at levels of 0, 10, 50, 200 or 500 mg/kg/day. A decrease in body weight gain was evident in both sexes at the high dose level. Male rats receiving both the 500 and 200 mg/kg/day diets showed an increase in kidney weight as well as grossly observable lesions. Females were not similarly affected at either dose level. Other effects included a trend toward increased liver weight in both sexes and the occurrence of gross liver lesions in the high-dose males. A NOAEL of 50 mg/kg/day is indicated under the parameters of this study.

Additional evidence for the renal toxicity of HCE was provided by a 16-day study which was conducted in Fischer-344 rats as part of the National Toxicology Program (NTP) investigation of this compound (NTP, 1989). Groups of five rats of each sex were administered twelve doses of 0, 187, 375, 750, 1,500 or 3,000 mg/kg HCE in corn oil by gavage over the 16-day period. The animals were observed twice daily and weighed weekly. After sacrifice, necropsy was conducted for all animals. Clinical signs of compound toxicity, including dyspnea, lacrimation, ataxia and prostration, were seen in the three highest dose groups while all of the animals in the two highest dose groups died before the termination of the study (NTP, 1989). In the 750 mg/kg dose group, 1/5 males and 2/5 females died prior to sacrifice; the surviving animals had body weights which were 25% lower than the controls for the males and 37% lower for the females. Hyaline droplets were identified in the cytoplasm of the renal tubular epithelium in all the males; tubular cell regeneration was apparent in males at doses of 187 and 375 mg/kg. Comparable effects were not observed in the females.

a. Skin and Eye Irritation, Dermal Sensitization

There was no primary skin irritation to the intact or abraded skin of six New Zealand White rabbits at 24 or 72 hours after the application of 0.5 g of the dry technical grade HCE for 24 hours. When 0.5 g of the technical grade HCE was applied as a paste in 0.5 mL of distilled water, no edema but a barely perceptible erythema was seen on the intact skin at 24 hours. On the abraded skin, moderate to slight erythemic reactions were seen while one rabbit showed a barely perceptible edema, indicating that HCE produced a mild skin irritation (Weeks and Thomasino, 1976).

Evidence of a very slight degree of epithelial hyperplasia was seen in the skin of rabbits exposed for 31 days to 20 applications of a saturated solution of HCE in 95% ethanol (<10% solution). The irritation was grossly evidenced by a slight hyperemia of the ear with scaliness and some faint follicular enlargement (Adams, 1937). This concentration produced no visible irritation when applied to the abdomen for 4 days. When slightly less than 1.5% of HCE in propylene glycol was applied 21 times to the rabbit ear over a period of 34 days, no irritation was evident. However, this same solution held in contact with the abdomen for the same period of time produced a faint hyperemia and exfoliation after three days which persisted unchanged throughout the

34 days of exposure. When the HCE was applied to rabbit skin as solutions in either Diamond paraffin oil or Deo base oil, severe irritation was produced but was largely attributed to the vehicle itself. Based on this study, HCE was not considered to be a particular skin hazard for man.

Moderate corneal opacity along with iritis and severe swelling and discharge were evident in five of six male New Zealand White rabbits exposed to a single application of 0.1 g of the dry technical grade HCE applied to the cornea and allowed to remain in contact overnight (Weeks et al., 1979). The irritation was no longer evident after 72 hours.

No dermal sensitization was indicated in male Hartley strain guinea pigs at 24 and 48 hours after a single challenge dose of HCE. The challenge dose was administered two weeks after the completion of a series of ten intradermal injections of 0.1 ml of a 0.1% solution of HCE in saline (Weeks and Thomasino, 1976). Hexachloroethane is not considered a sensitizing agent.

Weeks and Thomasino (1976) calculated a dermal LD_{50} of ≥32 g/kg for male New Zealand White rabbits (4/group) treated with a single dermal application of granular HCE mixed into a paste with equal quantities of distilled water. The doses, ranging from 1 g/kg to 32 g/kg, were applied to the shaved skin for a period of 24 hours. Signs of toxicity included weight loss but no skin irritation was evident. Two deaths occurred at the 32 g/kg level at 4 days after treatment. Gross examination of the surviving animals revealed a congestion of the cortex of the kidney in two rabbits and a hyperemia of the gastric mucosa in one treated at the highest dose level.

b. Six-week Studies

In an effort to determine the maximum tolerated dose for HCE, NCI (1978) conducted two subchronic toxicity studies utilizing Osborne-Mendel rats (5/sex/group) and $B6C3F_1$ mice (5/sex/group). The HCE was administered by gavage 5 days/week for 6 weeks followed by a 2-week observation period. The rats were administered doses of HCE in corn oil at levels of 0, 178, 316, 562, 1,000 or 1,780 mg/kg/day while the mice received doses of 0, 316, 562, 1,000, 1,780 or 3,160 mg/kg/day. Food and water were available ad libitum. Body weights were recorded weekly and the animals were observed for signs of toxicity. Mortality was recorded. No other parameters were studied in this experiment.

In the rat study, all animals receiving the high dose of 1,780 mg/kg/day died before the end of the 8-week experimental period while all rats at the middle dose level of 562 mg/kg/day survived. Body weights were comparable to controls at doses of ≤316 mg/kg/day while 38% of the males and 18% of the females developed a body weight depression at the 1,000 mg/kg/day level.

In the mouse study, survival remained at 100% for males up to 1,000 mg/kg/day and for females up to 1,780 mg/kg/day. At the high dose level of 3,160 mg/kg/day, mortality was 80% for males and 60% for females. Mean body weight gain was generally comparable to controls at doses up to

1,000 mg/kg/day and was substantially depressed at the high dose level. A Lowest-Observed-Adverse-Effect-Level (LOAEL) or NOAEL for this study would not be appropriate due to an insufficient number of parameters to judge toxicity.

c. Inhalation Toxicity

No toxic signs were evident in a group of six male rats exposed at room temperature (23°C) to a nominal concentration of 2.5 mg/L (260 ppm) of HCE for a period of 8 hours. Body weight and organ/body weight ratios did not differ significantly from controls and no gross or histopathological lesions were evident 14 days after exposure (Weeks and Thomasino, 1976). Exposure at 50°C to a nominal concentration of 57 mg/L (5,900 ppm) for 8 hours resulted in severe toxicity that included death of two of the animals at 8 hours after exposure. Other toxic signs included staggering gait and reduced body weight gain as well as evidence of a subacute diffuse interstitial pneumonitis with vascular congestion, minimal to moderate in severity, in two of the four surviving rats. Exposure at this same temperature to 17 mg/L (1,756 ppm) for 6 hours produced evidence of toxicity as indicated by a staggered gait and a reduced weight gain over the first 24 hours. No deaths occurred and no gross nor histopathological lesions were reported under these conditions. The dose of 17 mg/L (1,756 ppm) can be considered the LOAEL for a single inhalation exposure in rats.

Weeks et al. (1979) studied the effects of repeated exposure to vapor of HCE (≈99.8% pure) in 25 male and 25 female rats, 4 male dogs, 10 male guinea pigs, 20 male or female quail and 22 pregnant rats per exposure group. An additional 15 male rats were studied for behavioral effects following inhalation exposure. Exposure periods were for 6 hours/day, 5 days/week for 6 weeks and doses were analyzed at 0, 15, 48 or 260 ppm of the HCE vapor (equivalent to ≈ 0, 145, 465 or 2,515 mg/m^3). All animals were weighed weekly and observed daily for signs of toxicity. Toxicological evaluation was made for histopathological changes (rats, dogs, guinea pigs and quail), behavioral effects (rats), signs of sensitization (guinea pigs) and teratological effects (rats). Half of the treated animals were sacrificed at the end of the 6-week exposure period with the remaining animals sacrificed 12 weeks after termination of the exposure study.

At the 260 ppm exposure level, Weeks et al. (1979) reported toxic signs in dogs which included tremors, ataxia, hypersalivation, severe head bobbing and facial muscle fasciculation. During the exposure period, the dogs kept their eyelids closed and one dog convulsed, with death occurring after 5 hours of exposure (25% mortality). The signs recurred intermittently from Day 1 throughout the 6-week exposure period with recovery usually occurring overnight. Exposure at this level had no effect on the body weight of the dogs nor on the blood parameters measured (not specified). No exposure related lesions were observed upon histological examination.

The guinea pigs exposed at this 260 ppm dose level showed a significant reduction in body weight gain after one week of treatment and two guinea pigs died during each of the fourth and fifth weeks of treatment (40% mortality) (Weeks et al., 1979). A significant increase in liver/body weight ratio was

recorded. No sensitization response could be elicited and no histological lesions were observed.

Toxic signs in rats exposed to the high dose of 260 ppm of HCE vapor included tremors, ruffled pelt and the appearance of a red exudate around the eyes (Weeks et al., 1979). These signs appeared during the fourth week of exposure when two animals were found dead (4% mortality). Male rats showed a decrease in body weight gain beginning after 2 weeks of exposure. A significant increase in organ to body weight ratios was seen in the kidney, spleen and testes of males and in the liver of females. No gross changes were evident at necropsy. Comparison of the grouped data from a series of oxygen consumption studies indicated that exposure to 260 ppm of HCE vapor for 6 weeks resulted in a significant decrease in oxygen consumption. While nonspecific, this test was considered indicative of a lowered basal metabolic rate, possibly resulting from the prolonged inhalation of this upper respiratory irritant. Histological examination revealed an increased incidence and severity of mycoplasma-related lesions in the nasal turbinates, trachea and lungs of these exposed rats. The lesions were subclinical and consisted of a mucopurulent nasal exudate, lymphoid hyperplasia in the lamina propria of the trachea and pneumonitis, only rarely associated with bronchiectasis.

Pregnant female rats exposed to HCE vapor at the 260 ppm level on Days 6 through 16 of gestation developed slight to moderate tremors from Days 12 through 16 of gestation and body weight gain was significantly lower than controls starting on Day 8 of gestation (Weeks et al., 1979). At the 48 ppm exposure level, weight gain was significantly decreased beginning on Day 14 of gestation. No significant skeletal or soft tissue anomalies were detected in fetuses. All other maternal and fetal parameters, including a number of corpora lutea, implantation sites, resorption sites and viable fetuses as well as appearance, sex, weight and body length of viable fetuses, were within normal limits. Upon histological examination of the dams, an increased incidence of mucopurulent nasal exudate was seen in the 260 ppm (100% of the animals) and 48 ppm (85%) dosage groups when compared to controls.

Exposure to HCE vapor (0, 15, 48 and 60 ppm) caused no toxic effects in quails and no animals died after exposure (Weeks et al., 1979). Upon histological examination an excess of mucus, without evidence of inflammatory cells, was seen in the nasal turbinates in 20% of the high-dose group. This finding was considered a transient effect, as evidenced by lack of tissue damage, and due to direct exposure to the test compound.

Animals of all species sacrificed at 12 weeks after termination of the exposure studies had body weights comparable to controls (Weeks et al., 1979). No significant changes were seen upon gross necropsy and all toxic signs had disappeared. Incidence of upper respiratory irritations were comparable to controls.

Exposure to HCE vapor had no effect on the behavior of rats as measured by avoidance latency and spontaneous motor activity when compared to controls (Weeks et al., 1979). Pulmonary function studies conducted in dogs indicated

that inhalation of HCE had no effect on flow, tidal volume, transpulmonary pressure, compliance or resistance. Mean values for pre- and post-test compliance and resistance functions were comparable and were within normal limits.

At the 15 and 48 ppm exposure levels, no toxic signs were evident in any of the exposed groups except for the decreased weight gain in the pregnant dams. Body and organ weights were comparable to controls and no treatment-related changes were seen upon gross examination. A NOAEL of 48 ppm ($\approx$465 mg/m^3) for exposure to HCE vapor for six weeks can be established for all species (Weeks et al., 1979).

2. Longer-term Exposure

Studies were conducted in male and female rats fed HCE in the diet for 13 or 16 weeks, as well as in lifetime studies in rats and mice orally gavaged with HCE. Kidney toxicity was the most consistent finding of these studies although some non-neoplastic lesions were evident in the livers of the rats. The data from the lifetime studies indicate that HCE produces hepatocellular carcinomas in mice and renal cell carcinomas in rats.

a. Thirteen-week Studies

A 13-week study of HCE was conducted as a portion of the National Toxicology Program investigation of this compound (NTP, 1989). Groups of 10 male and 10 female Fischer-344 rats were given daily doses of 0, 47, 94, 188, 375 or 750 mg/kg HCE in corn oil by gavage. The animals were observed twice daily and weighed weekly. Following sacrifice, all animals were necropsied; key organs were weighed and the tissues of the controls, the high-dose group females, the penultimate-dose group males, and five of the high-dose group males were subjected to histopathological examination. Urine samples were collected from all animals at the end of the study.

Kidney changes were apparent in the males from all dose groups evaluated (NTP, 1989). The severity of the kidney damage increased with the dose. In five of the male rats which died prior to the completion of the study, there was renal papillary necrosis and degeneration accompanied by necrosis of the tubular epithelium. Hyaline droplets in the epithelial cytoplasm, tubular regeneration, and tubular casts were seen in these animals, as well as the males in the penultimate dose group. Urine specimens also contained renal casts. None of these manifestations of renal toxicity were seen in the females.

Some hepatocellular necrosis was seen in the males and females of the 375 and 750 mg/kg dose groups and in females from the 188 mg/kg dose group (NTP,1989). Both the males and females of the 750 mg/kg dose group had statistical increases in the relative weights of liver, heart, kidney and brain. Body weights were depressed in the males and females of the high-dose group.

An increased sensitivity of the male to kidney damage from HCE was apparent in the results of this study. The LOAEL for kidney effects in the male was 47 mg/kg; there was no corresponding NOAEL. In the females, the NOAEL was 94 mg/kg with a LOAEL of 188 mg/kg, based on the occurrence of hepatocellular necrosis (NTP,1989).

b. Sixteen-week Studies

Gorzinski et al. (1980b) conducted a 16-week feeding study in male and female CDF Fischer 344 rats (10/sex/group) in which the HCE (99.4% pure) was incorporated into the diet at target dose levels of 0, 3, 30 and 100 mg/kg/day (actual dose levels were analyzed to be approximately 0, 1.3, 20 and 82 mg/kg/day). Due to an apparent sublimation of the HCE in the feed during mixing, diets were changed daily and were made available to the animals for approximately 17 hrs/day (3 PM to 8 AM) with water available ad libitum. Further analysis of the eating pattern of the rats along with measurement of the time-related loss of HCE from the diets led to a conservative estimate of dietary intakes of 0, 1, 15 or 62 mg/kg/day (Gorzinski et al., 1985). Body weights were recorded weekly and food consumption was measured for one to two nonconsecutive days/week. The animals were observed for signs of toxicity on a twice-weekly basis. Hematological evaluations were conducted at approximately three months. Urinalyses were done after two months and three months on the test diets. Clinical chemistry parameters were measured at termination and included BUN, SGPT, creatinine and alkaline phosphatase. After 109–110 days on the test diets the animals were sacrificed by decapitation following an overnight fast. Terminal body and organ weights were recorded, blood was drawn for chemical evaluations, and the eyes were examined by fluorescent light illumination and abnormalities were recorded. The animals were examined for gross abnormalities and all major organs were prepared for histopathological examination. The liver, kidney and adrenals of all test animals were microscopically evaluated while the remaining tissues from only the control and high-dose groups were examined.

No signs of toxicity were noted and no deaths occurred during the course of this feeding study. Body weight gain and food intake levels were comparable between control and test groups. Clinical chemistry parameters were generally comparable with the only significant alteration being a very slight decrease in the BUN in the male rats receiving the lowest dose level. Hematological determinations and routine urinalyses were comparable to controls. Total urine volume was increased in both sexes at all dose levels but not in a dose-related manner. Female rats showed no difference in excretion of urinary creatinine, porphyrins or delta-aminolevulinic acid (delta-ALA) while total creatinine excretion was elevated in all treated males at the 3-month evaluation. Excretion of coproporphyrin was unaffected in the males but the uroporphyrin excretion was increased in males receiving the two highest doses as measured during both evaluation periods. Delta-ALA was increased in all treated males but was not dose-related at the 60-day evaluation.

Upon necropsy, the kidneys of the male rats receiving the high-dose diet were pale and mottled and the absolute and relative kidney weights were significantly increased. Only one female from the high-dose group displayed a mottling of the kidney and this change was considered equivocal. The absolute

and relative liver weights were significantly increased in the high-dose males while only the relative liver weights were significantly increased in females at this dose level.

Histopathological changes in the kidneys of the high-dose males included an increased, slight to moderate, renal tubular atrophy and degeneration with or without peritubular fibrosis, increased renal tubular cytoplasmic clumping and droplet formation, also slight to moderate in degree, and scattered or isolated renal tubules with slight hypertrophy and/or dilation of the proximal convoluted tubules. Similar lesions, less severe in degree, were evident in the 30 mg/kg/day test males. In female rats receiving the 100 mg/kg/day dose, the kidney alterations were limited to an increased incidence of very slight renal tubular atrophy and degeneration.

Liver lesions were seen in the high-dose males and consisted of an increased incidence of slight swelling of the hepatocytes. A similar effect was considered equivocal in the males receiving the 30 mg/kg/day diet. No other significant changes were seen in the tissues examined.

Evaluation of the results of this study indicate that the kidney is the target organ for HCE toxicity and that the male rats are more sensitive to this effect than the females. Based on this study, a NOAEL of 1.3 mg/kg/day (the analyzed low dose) is indicted for kidney toxicity in rats.

c. Lifetime Exposure

The National Cancer Institute (NCI, 1978) conducted a 78-week cyclic oral intubation study in 50 male and 50 female Osborne-Mendel rats followed by a treatment-free observation period of 34 weeks for the purpose of developing a database for the carcinogenic evaluation of HCE. The rats received HCE in corn oil at a low and high dose of 250 and 500 mg/kg/day, 5 days per week for a period of 22 consecutive weeks, followed by a 1-week, treatment-free interval. Thereafter, until the end of the 78 weeks, the rats were intubated for 4 consecutive weeks followed by 1 treatment-free week, in a cyclical pattern, for a total of 66 weeks of HCE treatment. Based on this schedule, time-weighted average doses for the 78-week period were reported to be 212 and 423 mg/kg/day. Two control groups consisting of 20 rats/sex/group were either untreated or received corn oil by gavage. Vehicle control rats were started six weeks prior to the remaining groups and were approximately two weeks older (8 weeks vs 6 weeks) when receiving their first intubation. The rats were housed in individual cages and food and water were available ad libitum. Body weights and food consumption were recorded weekly over the first 10 weeks and at monthly intervals through the end of the study. The animals were observed for signs of toxicity, behavioral changes and tissue masses and mortality was recorded. At the end of the 112-week study, surviving animals were euthanized by exsanguination under anesthesia and all major organs, as well as any gross lesions, were subjected to both a gross and microscopic examination. All animals dying or sacrificed moribund during the course of this study were similarly examined.

Toxic signs, generally comparable at both dosage levels during the first year of the study, included a hunched appearance, reddened, squinted or lacrimating

eyes and urine staining of the abdomen. High-dose females, beginning at Week 4 through termination, had a higher frequency of the abdominal urine staining. During the second year of the study, the appearance and behavior of the animals in all groups generally were comparable. Mean body weights of the treated males were depressed in a dose-related manner while only the high-dose females showed signs of a slight body weight depression. Respiratory abnormalities and signs of aging were comparable between all test and control groups. Toxic signs such as transient tremors, ataxia and vaginal discharge, seen in several treated animals, were considered isolated and spontaneous.

Significant mortality was noted in the treated males with survival to 90 weeks being 48 and 38% for the low and high dose, respectively, as compared to 70 and 55% for the untreated and corn oil-treated controls, respectively. Similar findings of significant dose-related mortality were reported for the females with survival to the end of the study (112 weeks) being 70% for both control groups as compared to 54 and 48% for the low and high doses, respectively.

Significant histopathological lesions in the treated animals were limited to the kidney and were described as a toxic tubular nephropathy characterized by degeneration, necrosis and the presence of large hyperchromatic regenerative epithelial cells. Chronic interstitial nephritis and fibrosis, focal pyonephritis, tubular ectasia, cast formation and focal glomerulosclerosis were also reported. Four low-dose male rats also developed renal tubular-cell adenomas but the incidence was not statistically significant. The incidence of toxic nephropathy in the male rats was 45% (22/49) and 66% (33/50) for the low and high doses, respectively, and 18% (9/50) and 59% (29/49) for the low- and high-dose females, respectively. No control rats developed this lesion. Ten percent of the high-dose male rats (3/29) also showed an increase in the incidence of interstitial cell tumors of the testes which were first observed at 109 weeks. This incidence was significant by the Cochran-Armitage test but not under the parameters of the Fisher exact test. No significant neoplastic lesions were reported in the rat study. Significant pathology and mortality at both dose levels in the male rats precluded the development of a NOAEL or LOAEL for HCE in rats chronically exposed by oral intubation.

Chronic toxicity studies were also conducted by NCI (1978) in 50 male and 50 female $B6C3F_1$ mice intubated orally with HCE in corn oil at initial levels of 500 and 1,000 mg/kg/day for 8 weeks with these doses increased to 600 and 1,200 mg/kg/day, respectively, for the remaining 70 experimental weeks. A time-weighted average dose of 590 and 1,179 mg/kg/day for the low and high doses, respectively, was reported. The animals were dosed 5 days per week for a total of 78 weeks, followed by an additional 13-week observation period without treatment. Two control groups (20/sex/group) were included, one group receiving corn oil by gavage while the remaining control group remained untreated. The untreated controls were started on the experiment approximately 6 weeks prior to the orally dosed animals. All other experimental parameters and observation intervals were the same as in the rat study.

No specific signs of toxicity were observed in the treated mice over the first 34 weeks of treatment. Thereafter, a greater frequency of hunched or thin

appearance was seen in the treated groups. The incidence of palpable nodules was also slightly increased in the treated groups. Variations in body weight gain were not dose-related. No significant relationship was seen between dose and mortality rate. Survival of male control groups was unexpectedly low with rates of 25 and 5% for the vehicle and untreated controls, respectively, as compared to 14% for the low-dose males and 58% for the high-dose males. Survival to the end of the experiment among female mice was 85% for untreated controls, 80% for low-dose females and corn oil controls and 68% for the high-dose females.

Non-neoplastic lesions in the mouse experiment included a toxic nephropathy which was comparable between both sexes and both treatment groups with an occurrence rate between 92 and 100%. The lesion was characterized by degeneration of the convoluted tubule epithelium at the cortical-medullary junction. Hyaline casts were occasionally present and, in some cases, the damaged cells were replaced by enlarged dark-staining regenerative tubular epithelium. Infiltration of the inflammatory cells, fibrosis and calcium deposition was often seen.

A statistically significant incidence of hepatocellular carcinomas was seen in both the male and female high and low dose groups when compared to the matched vehicle control group from this study or a pooled vehicle control groups from other concurrent studies. Details on carcinogenicity are presented in Section VI.B.5. (Carcinogenicity). Due to the occurrence of hepatocellular carcinoma, as well as non-neoplastic toxic nephropathy in both sexes at both dose levels, neither a NOAEL nor a LOAEL can be determined for mice orally exposed to HCE over their lifetime.

In an effort to collect data concerning the toxicity of HCE to the kidney and to examine the carcinogenicity of this chemical, the NTP carried out a 2-year bioassay using groups of 50 male and 50 female Fischer-344 rats (NTP, 1989). The animals were dosed by gavage for 5 days per week over a 2-year period. The doses administered to the males and females differed due to the difference in their sensitivity to HCE as determined in shorter duration studies. The male rats received doses of 0, 10 or 20 mg/kg while the females received doses of 0, 80 or 160 mg/kg. The animals were observed twice daily; initial weighings were conducted weekly but decreased to monthly measurements as the duration of the study increased. Following sacrifice, all the animals were necropsied. The tissues of the controls and high-dose animals were subjected to histopathological examination. Selected tissues, including the kidney, were examined from the low-dose group animals.

In this 2-year bioassay, HCE had minimal effects on the body weights of the exposed animals (5-9% lower than vehicle controls) and their longevity (NTP, 1989). There was a dose-related increase in the severity of nephropathy and hyperplasia seen in the male rats. The occurrence of nephropathy was also increased in the female rats. Tubular cell degeneration, dilation, atrophy, inflammation and regeneration were apparent in both sexes but were more severe in males. Damage to the renal papillae and pelvic transitional epithelium occurred in the males. There was a dose-related increase in the combined incidence of adenomas and carcinomas in the males with an occurrence of 1 for the controls, 2 for the low-dose group and 7 for the high-dose group.

Adenomas or carcinomas were not present in exposed females. Pheochromocytomas of the adrenal gland were also present in some of the males. The details on the carcinogenicity of HCE are discussed in Section VI.B.5 (Carcinogenicity). Due to the occurrence of carcinomas in the males, this study is not appropriate as the source of a NOAEL or LOAEL for long-term chronic exposure to HCE.

NTP hypothesized that the increased sensitivity of males in the renal toxicity of HCE was a product of the accumulation of α_{2u}-globulin in hyaline droplets in the tubular epithelial cells. α_{2u}-Globulin is a protein which is synthesized by the liver and secreted into the blood. It is then, apparently, filtered through the glomeruli and partially reabsorbed through the proximal tubules. In the presence of HCE, as well as several nonpolar hydrocarbons such as decalin and gasoline, α_{2u}-globulin accumulates in hyaline droplets in the kidney cells. Based on limited evidence, α_{2u}-globulin is an excretory protein in male but not female rats. This may explain the male susceptibility to kidney damage from HCE.

3. Reproductive Effects

No information was found in the available literature regarding the reproductive effects of HCE in animals.

4. Developmental Effects

Weeks et al. (1979) conducted teratology studies in pregnant female Sprague-Dawley rats (22/group) given oral doses of HCE (≈99.8% pure) by gavage from Day 6 through Day 16 of gestation at levels of 0, 50, 100 or 500 mg/kg/day. All animals were sacrificed by intracardiac injection of sodium pentobarbital on Day 20 of gestation. Parameters examined included number of corpora lutea, implantation sites and resorption sites. Viable fetuses were examined for gross abnormalities and the sex, weight and body length were recorded. All grossly abnormal fetuses along with eight normal fetuses per dam were further examined for soft tissue abnormalities and skeletal malformations.

Pregnant females receiving the high dose of 500 mg/kg/day demonstrated a significant reduction in body weight gain beginning at Day 8 of gestation. Significantly lower gestation indices were recorded among the fetuses from the high-dose exposure group. The number of viable fetuses per dam were also reduced while a higher fetal resorption rate was recorded. No significant skeletal or soft tissue anomalies were seen in any of the HCE-exposed fetuses. Maternal and fetal parameters were comparable to controls in the two lower exposure groups. Histological examination of the dams revealed an increased incidence (70%) of upper respiratory tract infection with subclinical pneumonitis evident in 20%. Only 10% of control dams were similarly affected. Based on this study, HCE is not a teratogenic agent at exposure levels up to 500 mg/kg/day. The high dose level was maternally toxic and resulted in a slowing of fetal development. A NOAEL for maternal and fetotoxic effects of 100 mg/kg/day is indicated.

5. Carcinogenicity

In a study conducted by NCI (1978) in Osborne-Mendel rats and B6C3F_1 mice orally intubated with HCE in corn oil, a statistically significant incidence of hepatocellular carcinoma was reported in the mice. The mice were dosed 5 days/week for a period of 78 consecutive weeks with an untreated observation period of an additional 13 weeks to yield time-weighted average doses of 590 and 1,179 mg/kg/day in the low- and high-dose mice, respectively. Details on the parameters for both experiments are contained in Section VI.B.2.b. (Lifetime Exposure). Due to an unusually high mortality rate among the male control groups, the incidence was statistically compared against both the vehicle control group from this study as well as a pooled vehicle control group from several concurrent studies. The incidence was significant by the Cochran-Armitage test ($p<0.001$) for both sexes against both controls and by the Fisher exact tests ($p<0.008$) for both sexes as compared to the pooled controls. The hepatocellular carcinomas varied greatly in appearance with some containing well-differentiated hepatic cells with a relatively uniform arrangement of cords and others containing anaplastic cells with large hyperchromatic nuclei often containing inclusion bodies with vacuolated pale cytoplasm. The neoplastic liver cells varied in arrangement from short, stubby cords to nests of hepatic cells. Occasionally a pseudo-acinar formation was seen and mitotic figures were often present. Incidence figures for the occurrence of hepatocellular carcinomas in the mouse study are contained in Table VI-1. No treatment-related neoplasms were reported in the rat study, although an increased incidence of interstitial cell tumors of the testes was reported in 10% of the high-dose male rats. The incidence (3/29) was significant by the Cochran-Armitage test but not under parameters of the Fisher exact test.

Lifetime exposures to HCE resulted in renal carcinomas and adenomas in Fischer-344 rats during a bioassay conducted by NTP (1989). Groups of 50 males and 50 females were dosed with HCE in corn oil by gavage for 5 days per week over a 2-year period. The doses administered to the males and females differed due to a difference in their renal sensitivity to HCE. The male rats received doses of 0, 10 or 20 mg/kg while the female doses were 0, 80 or 160 mg/kg. Following sacrifice all the animals were necropsied. The tissues of the controls and high-dose animals were subjected to histopathological examination. Selected tissues, including the kidney, were examined from the low-dose group animals. There was a dose-related increase in the combined incidence of adenomas and carcinomas in the males with an occurrence of 1 for the controls, 2 for the low-dose group and 7 for the high-dose group. Carcinomas were only identified in the high-dose group (3/50). Each carcinoma was apparent on gross examination of the organ; one carcinoma had metastasized. The number of tumors seen in the high-dose group was significantly greater than that for both the concurrent controls ($p>0.03$, Fisher Exact Test) and the historical controls. The historical incidence of renal tubular cell adenomas is 1/300. The exposed females were not affected. Pheochromocytomas of the adrenal gland were also present in some of the males. The renal lesions were considered by NTP to be indicative of compound carcinogenicity while the pheochromocytomas were judged to be supportive evidence for carcinogenicity. On the basis of these data, NTP concluded that there was clear

evidence of carcinogenicity for HCE in the male rat and no evidence of carcinogenicity in female rats.

6. Genotoxicity

Simon and Kauhanen (1978) tested HCE dissolved in DMSO in the standard Salmonella/microsome assay (strains TA1535, TA1537, TA1538, TA98 and TA100) at concentrations ranging from 10 to 10,000 ug/plate, with and without addition of a metabolic activation system (S-9 fraction). No mutagenic response was obtained at any concentration. A slight toxicity was seen at the 10,000 ug/plate concentration without metabolic activation. When tested with Saccharomyces cerevisiae D3 at a dose range of 0.1 to 5.0%, no dose-related increase in the number of mitotic recombinants was seen.

No evidence of mutagenicity was seen in a series of in vitro microbial assays conducted by Weeks et al. (1979). Hexachloroethane was tested at doses ranging from 0.1 to 500 ug/plate, with and without metabolic activation with liver enzyme preparation, in Salmonella typhimurium (strain TA-1535, TA-1537, TA-1538, TA-98 and TA-100) and in S. cerevisiae (strain D4).

Nakamura et al. (1987) tested HCE for its potential to induce DNA damage in a newly developed test which is based on the ability of a chemical to induce umu gene expression, as measured through cellular beta-galactosidase activity. This study utilized a new S. typhimurium tester strain, TA1535/pSK1002, in which an umuC-lacZ fused gene had been introduced. The ability for HCE to induce a genotoxic response was measured by the beta-galactosidase activity produced by the fused gene. When tested at concentrations up to 42 ug/mL, with and without metabolic activation (S9), HCE was considered nongenotoxic.

Table VI-1 Incidence of Hepatocellular Carcinoma in $B6C3F_1$ Mice Orally Dosed with HCE for up to 78 Weeks[a]

Dose	Male	Female
590 mg/kg/day	15/50[b]	20/50[b]
1,179 mg/kg/day	31/49[b]	15/49[b]
Pooled vehicle control[c]	6/60	2/60
Matched vehicle control	3/20	2/20
Untreated control	1/18	0/18

Reference: NCI, 1978

[a] Number with lesion/number examined.
[b] Significant ($p < 0.001$) by the Cochran-Armitage test, when compared with either the matched or pooled vehicle control.
[c] Combined controls from HCE and two additional concurrent studies.

Table VI-2 Incidence of Renal or Renal Tubule Lesions in Male Rats Orally Dosed with HCE for Two Years

	Vehicle Control	10 mg/kg	20 mg/kg
Hyperplasia	2/50	4/50	11/50
Adenoma	1/50	2/50	4/50
Carcinoma	0/50	0/50	3/50
Adenoma or carcinoma	1/50	2/50	7/50

Reference: NTP, 1989

VII. HEALTH ADVISORY DEVELOPMENT

Available data on the toxic effects of HCE in several species, for periods ranging from single dose oral LD_{50}s to continuous exposure studies in the diet of rats (16 weeks), have been evaluated. Carcinogenic studies in rats and mice, developmental toxicity studies in rats and genotoxicity assays are also included. Studies on the reproductive effects of HCE exposure are not available. Exposures to HCE by inhalation are reported for several species. Human exposure data is limited, with "irritation" the only effect reported following industrial exposure.

Toxic effects of HCE (12 days to 16 weeks) are generally related to its effects on the kidney, although liver toxicity has also been indicated in several studies (Weeks et al., 1979; Gorzinski et al., 1980a,b; NTP, 1989). In rats and sheep, acute exposure has resulted in CNS effects which include incoordination, tremor, ataxia and signs of gasping (Fowler, 1969; Weeks and Thomasino, 1976). Exposure by inhalation resulted in an increased incidence of mucopurulent exudate in rats and quail, and CNS-related effects in rats and dogs (Weeks et al., 1979). Lifetime administration by oral intubation to rats and mice resulted in a significant increase in neoplastic lesions in the livers of mice (NCI, 1978). Skin and eye irritation studies indicated that HCE is a mild irritant in rabbits (Weeks and Thomasino, 1976; Weeks et al., 1979), but no dermal sensitization was demonstrated in guinea pigs (Weeks and Thomasino, 1976). Dermal toxicity is low.

Indications of the toxic effects of HCE in the kidneys were first seen by Weeks and Thomasino (1976) in the cortex of a male rat that died while part of an LD_{50} study. A non dose-related toxic tubular nephrosis was reported in New Zealand white rabbits following 12 days of oral intubation with HCE at levels of 320 and 1,000 mg/kg/day (Weeks et al., 1979). Increased kidney weight and evidence of gross lesions were seen in male rats following 16 days of feeding with HCE at 200 and 500 mg/kg/day. Females were not similarly affected (Gorzinski et al., 1980a). In 1980(b), Gorzinski et al. reported the development of pale and mottled kidneys, with a significant increase in absolute and relative weight, in male rats fed HCE for 16 weeks at a level of 100 mg/kg/day. The histological changes were described as slight to moderate renal tubular atrophy and degeneration, slight to moderate tubular cytoplasmic clamping and droplet formation, and scattered to isolated hypertrophy and/or dilatation of the proximal convoluted tubule. Similar lesions, less severe in degree, were also reported in the rats fed at the 30 mg/kg/day test level. These levels were analyzed to contain actual amounts of 82 and 20 mg/kg/day, respectively. High dosed females exhibited only a very slight renal tubular atrophy and degeneration.

No kidney lesions were seen following inhalation exposure of dogs, rats, guinea pigs and quail at levels up to 260 ppm (equivalent to approximately 2,515 mg/m^3). Exposure duration was for 6 hours/day, 5 days/week for 6 weeks.

In a lifetime bioassay in rats and mice, NCI (1978) reported the development of histopathological lesions in the kidneys of rats orally dosed at a time-weighted average of 212 and 423 mg/kg/day. Exposure by oral intubation took

place 5 days/week in a cyclic manner with 4 weeks of dosing followed by 1 treatment-free week for a total of 78 weeks. The lesions were described as those found in toxic tubular nephropathy and were evident in both sexes at both dose levels. They were characterized by signs of degeneration, necrosis, and the presence of large hyperchromatic regenerative renal cells. Chronic interstitial nephritis, fibrosis, focal pyonephritis, tubular ectasia, cast formation and focal glomerulosclerosis were also seen. Renal tubular cell adenomas were reported in four of the low-dose male rats, but the incidence was not statistically significant.

Similar findings of nephropathy were seen in male and female rats during a 2-year bioassay of HCE (NTP, 1989). The males received doses of 0, 10 and 20 mg/kg in corn oil by gavage 5 days per week for a lifetime; the corresponding doses for females were 0, 80 and 160 mg/kg. In this study, the incidence of renal adenomas plus carcinomas in the males was significantly greater than in the controls; the non-neoplastic damage to the male kidneys was also more pronounced than that seen in the females.

Toxic nephropathy was also reported in mice treated with time-weighted average doses of 590 and 1,179 mg/kg/day over 78 weeks of continuous exposure. The incidence, 92 and 100% for the low and high dose, respectively, was comparable between sexes. Lesions were characterized by a degeneration of the epithelium of the convoluted tubules. Calcium deposition, fibrosis and infiltration of inflammatory cells was also seen.

Early indications of liver toxicity due to the oral administration of HCE were first seen in studies with sheep (Fowler, 1969). The sheep were dosed with HCE at 0.5 to 1.0 g/kg by drenching bottle. A significant increase in the plasma enzymes indicative of liver toxicity was seen 48 hours after treatment. Results of a BSP clearance test indicated that toxicity was due to a reduction in the excretory capacity of the liver. No changes in the chemical or function properties of liver microsomes were seen following a single oral dose of 6 g/kg in rats (Reynolds, 1972). When New Zealand white rabbits were administered HCE in methylcellulose at doses up to 1,000 mg/kg/day for 12 days, relative liver weights were significantly increased at the high dose level (Weeks et al., 1979), with a non-significant increase in alkaline phosphatase, SGOT and bilirubin. Coagulation necrosis, hemorrhage and fatty degeneration were seen upon histopathological examination of livers from the rabbits receiving doses of 320 and 1,000 mg/kg/day. Gross liver lesions were observed in male rats fed HCE in the diet at 500 mg/kg/day for 16 days with a trend toward increased liver weight apparent in both sexes (Gorzinski et al., 1980a). Feeding of HCE to rats at levels up to 100 mg/kg/day for a period of 16 weeks resulted in an increased incidence of a slight swelling of the hepatocytes at the highest dose level with equivalent changes at the next highest dose of 30 mg/kg/day (actual levels analyzed at 82 and 20 mg/kg/day, respectively; Gorzinski et al., 1980b).

No liver lesions were observed in dogs, rats, guinea pigs and quail exposed to HCE by inhalation at levels up to 260 ppm ($\approx$ 2,515 mg/m^3). Exposure was for 6 hours/day, 5 days/week for a period of 6 weeks. In a bioassay in rats and mice (NCI, 1978), no liver lesions were seen in rats orally dosed with HCE at

time-weighted average doses up to 423 mg/kg/day for 78 weeks. Mice similarly treated at time-weighted average doses of 590 and 1,179 mg/kg/day developed a statistically significant incidence of hepatocellular carcinomas. The lesions were seen at both dose levels and were significant when paired to both the matched vehicle controls and pooled vehicle controls from other studies.

An increased incidence of interstitial cell tumors of the testes was reported in 10% of the male rats orally dosed with HCE at a time-weighted average dose of 423 mg/kg/day for 78 weeks in a cyclical pattern (NCI, 1978). The incidence was significant by the Cochran-Armitage test but not the Fisher exact test.

No reproductive data were located in the available literature but developmental studies indicated that HCE is not teratogenic in rats. Exposure was both by oral intubation and by inhalation on days 6 through 16 of gestation. Doses were up to 500 mg/kg/day in the oral study and up to 260 ppm ($\approx$2,515 mg/m^3) in the inhalation study (Weeks et al., 1979). Indications of maternal as well as fetotoxic effects were seen in the oral study, as evidenced by a significant decrease in maternal body weight gain at Day 8, as well as a significant decrease in gestation indices. These included a decrease in the number of viable fetuses per dam and higher fetal resorption rate. Histological examination revealed an increased incidence of upper respiratory infection with subclinical pneumonitis. Significant decreases in maternal weight gain were seen in the inhalation study at both the 48 and 260 ppm levels. No other maternal or fetotoxic effects were affected by inhalation of HCE although maternal rats displayed an increase in the incidence of mucopurulent nasal exudate at the 260 ppm exposure level.

An increased incidence of mycoplasma-related lesions was also seen in male and female rats exposed to HCE vapor at 260 ppm for 6 hours/day, 5 days/week for 6 weeks. The lesions were subclinical and consisted of mucopurulent exudate affecting the nasal turbinates, trachea and lungs of the exposed rats. Excess mucus was also evident in the nasal turbinates of quail similarly exposed (Weeks et al., 1979).

No skin irritation was indicated in rabbits exposed to dry technical grade HCE, but a mild irritation resulted from exposure to a HCE paste (Weeks and Thomasino, 1976). No dermal sensitization was seen in guinea pigs. Dermal toxicity was mild with an LD_{50} of $\geq$32 g/kg reported. Moderate corneal opacity, as well as iritis and severe swelling and discharge, developed in rabbits exposed to a single application of dry HCE to the cornea.

A. Quantification of Toxicological Effects

Health Advisories are generally determined for One-day, Ten-day, Longer-term (approximately 7 years) and Lifetime exposures if adequate data are available that identify a sensitive noncarcinogenic end point of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$HA = \frac{(NOAEL \text{ or } LOAEL) \times (BW)}{(UF(s)) \ (__ \text{ L/day})} = ___ \text{ mg/L } (___ \text{ug/L})$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level in mg/kg bw/day.

BW = assumed body weight of a child (10 kg) or an adult (70 kg).

UF = uncertainty factor (10, 100 or 1,000), in accordance with NAS/ODW guidelines.

__L/day = assumed daily water consumption of a child (1 L/day) or an adult (2 L/day).

1. One-day Health Advisory

No data were located in the available literature that were considered suitable for the calculation of a One-day HA. It is suggested that the Ten-day HA of 5 mg/L for the 10 kg child be used as a conservative estimate for the One-day HA.

2. Ten-day Health Advisory

The study of Gorzinski et al. (1980a) has been selected to serve as the basis for developing a Ten-day HA for the 10 kg child. Two studies are of appropriate duration to be considered for development of a Ten-day HA. Upon gross examination, kidney lesions were seen in male rats fed HCE in the diet at 200 and 500 mg/kg/day for 16 days (Gorzinski et al., 1980a) and in New Zealand white rabbits orally dosed with HCE at 320 and 1,000 mg/kg/day for 12 days (Weeks et al., 1979). Liver degeneration and necrosis, as well as a significant decrease in body weight gain, were also seen in the rabbits at both treatment levels, while a significant decrease in weight gain and gross liver lesions were seen in the male and female rats at the 500 mg/kg/day level. The weight loss in rabbits receiving the 320 mg/kg/day dose developed on Day 10 of this 12-day study. Initial results of these studies indicate apparent NOAELs of 100 mg/kg/day in rabbits and of 50 mg/kg/day in rats. In view of the late developing decrease in body weight gain in the rabbit study, the possibility of this toxic sign also occurring over time in the rabbits at the 100 mg/kg/day level must be considered. In addition, the lack of histopathological data in the rat study warrants the use of caution in selection of an appropriate NOAEL. It is, therefore, recommended that the more conservative NOAEL of 50 mg/kg/day from the 16-day rat study be used to determine a Ten-day HA which is calculated as follows:

For the 10 kg child:

$$\text{Ten-day HA} = \frac{(50 \text{ mg/kg/day})\ (10 \text{ kg})}{(100)\ (1 \text{ L/day})} = 5 \text{ mg/L } (5{,}000 \text{ ug/L})$$

where:

50 mg/kg/day - NOAEL based on absence of effects on the liver, kidney and growth rate of rats exposed to HCE in the diet for 16 days.

10 kg - assumed weight of a child.

1 L/day - assumed water consumption by a 10 kg child.

100 - uncertainty factor; this uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from an animal study is employed.

3. Longer-term Health Advisory

The only study of longer duration in which a NOAEL which applied to both sexes could be determined was the 16-week study of Gorzinski et al. (1980b) in which rats were fed HCE in the diet at target levels of 0, 3, 30 or 100 mg/kg/day. An analysis of the diets indicated that actual levels of consumption were in the range of 0, 1.3, 20 and 82 mg/kg/day. Based on the occurrence of kidney lesions in the male rats at the two highest dose levels, a NOAEL of 1.3 mg/kg/day was indicated. There was no NOAEL for male rats in the NTP (1989) 13-week study in which kidney damage was apparent at doses of 47 mg/kg and greater. The kidneys of female rats were only mildly affected by HCE exposure at the highest dose level, and this change was considered equivocal. Liver lesions were also seen in the high-dose males with equivocal changes in the liver at the next lower level. Using the NOAEL of 1.3 mg/kg/day, the Longer-term HA is calculated as follows:

For the 10 kg child:

$$\text{Longer-term HA} = \frac{(1.3 \text{ mg/kg/day})\ (10 \text{ kg})}{(100)\ (1 \text{ L/day})} = 0.13 \text{ mg/L}$$

(rounded to 100 ug/L)

where:

1.3 mg/kg/day - NOAEL based on absence of kidney and liver lesions in rats exposed to HCE in the diet for 16 weeks.

10 kg - assumed weight of a child.

1 L/day - assumed water consumption by a 10 kg child.

100 - uncertainty factor; this uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from an animal study is employed.

For the 70 kg adult:

$$\text{Longer-term HA} = \frac{(1.3 \text{ mg/kg/day})\ (70 \text{ kg})}{(100)\ (2 \text{ L/day})} = 0.455 \text{ mg/L}$$

(rounded to 450 ug/L)

where:

1.3 mg/kg/day - NOAEL based on absence of effects on the kidney and liver of rats exposed to HCE in the diet for 16 weeks.

70 kg - assumed weight of an adult.

2 L/day - assumed water consumption by a 70 kg adult.

100 - uncertainty factor; this uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from an animal study is employed.

4. Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three-step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL is a medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The Lifetime HA is determined in Step 3 by factoring in other sources of exposure, the relative source contribution (RSC). The RSC from drinking water is based on actual exposure data or, if data are not available, a value of 20% is assumed. If the contaminant is classified as a Group A or B carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986), then caution should be exercised in assessing the risks associated with lifetime exposure to this chemical.

Hexachloroethane is classified in Group C: Possible Human Carcinogen, according to EPA's weight-of-evidence scheme for classification of carcinogenic potential. Because of this, caution must be exercised in making a decision on how to deal with possible lifetime exposure to this substance. The risk manager must balance this assessment of carcinogenic potential against the likelihood of occurrence of health effects related to noncarcinogenic end points of toxicity. In order to assist the risk manager in this process, drinking water concentrations associated with estimated excess lifetime cancer risks over the range of one in ten thousand to one in a million are provided in the following section. In addition, in this section, a Drinking Water Equivalent Level (DWEL) is derived. The DWEL and estimated cancer excess are determined for the 70 kg adult, ingesting two liters of water per day. Also provided is an estimate of the excess cancer risk that would result if exposure were to occur at the DWEL over a lifetime.

Neither the cancer risk estimates nor the DWEL take relative source contribution into account. The risk manager should do this on a case-by-case basis, considering the circumstances of the specific contamination incident that has occurred.

The 16-week study of Gorzinski et al. (1980b) has also been selected to serve as a basis for the Lifetime HA for HCE. The 78-week screening study by NCI (1978), using mice and rats, and the NTP (1989) lifetime study in rats failed to establish a NOAEL or LOAEL for HCE. A dose of 47 mg/kg/day (NTP, 1989) in rats and 590 mg/kg/day in mice (NCI, 1978) produced significant signs of toxicity in the liver and/or kidney of both species. In addition, a significant increase in the incidence of hepato-cellular carcinomas was reported at 590 mg/kg/day in the study in mice (NCI, 1978), and an increased incidence of renal neoplasms in male rats at a dose of 20 mg/kg/day (NTP, 1989). Therefore the NOAEL of 1.3 mg/kg/day (Gorzinski et al., 1980b) may be used to calculate the Lifetime HA as follows:

Step 1: Determination of Reference Dose (RfD)

$$\text{RfD} = \frac{(1.3 \text{ mg/kg/day})}{(1000)} = 0.0013 \text{ mg/kg/day}$$
(rounded to 1 ug/kg/day)

where:

1.3 mg/kg/day = NOAEL based on the absence of effects on the kidney and liver of rats exposed to HCE in the diet for 16 weeks.

1000 = uncertainty factor; this uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from a less-than-lifetime animal study was employed to derive an RfD.

Step 2: Determination of a Drinking Water Equivalent Level (DWEL)

$$\text{DWEL} = \frac{(0.001 \text{ mg/kg/day})\ (70 \text{ kg})}{(2 \text{ L/day})} = 0.035 \text{ mg/L}$$
(rounded to 40 ug/L)

where:

0.001 mg/kg/day = RfD

70 kg = assumed body weight of an adult

2 L/day = assumed daily water consumption of an adult.

Step 3: Determination of the Lifetime Health Advisories

$$\text{Lifetime HA} = \frac{(0.035)\ (0.2)}{10} = 0.0007 \text{ mg/L}$$
(rounded to 1 ug/L)

where:

0.2 - Relative Source Contribution

10 - Extra uncertainty factor to account for equivocal evidence of carcinogenicity, used in accordance with ODW Policy for Group C chemicals.

B. Quantification of Carcinogenic Potential

Applying the criteria described in EPA's guidelines for assessment of carcinogenic risk (U.S. EPA, 1986), HCE is classified in Group C: Possible Human Carcinogen. This category applies to agents for which there is inadequate evidence from human studies and sufficient evidence from animal studies.

The risk manager must balance the assessment of carcinogenic potential against the likelihood of occurrence of health effects related to noncarcinogenic end points of toxicity. In order to assist the risk manager in this process, drinking water concentrations, associated with cancer risks over the range of one excess tumor in populations of ten thousand (10^{-4}) to one excess tumor in populations of one million (10^{-6}) for the 70 kg adult, drinking 2 liters of water per day, are provided.

Technical grade hexachloroethane (98% pure) was administered by gavage to 50 each male and female Osborne-Mendel rats and B6C3F_1 mice (NCI, 1978). Rats were treated with either 250 or 500 mg HCE/kg bw/day, 5 days/week for 22 weeks. After this time animals were cyclically rested 1 week and gavaged for 4 succeeding weeks up to Week 78; an observation period of 33–34 weeks followed. TWA final treatment doses were 212 and 432 mg/kg bw/day. There was no evidence of hexachloroethane-induced neoplastic growth in rats.

The mice were administered 500 or 1,000 mg/kg/day, 5 days/week, continuously. At Week 9, the doses were increased to 600 and 1,200 mg/kg/day and this dosage was maintained until Week 78. The mice were observed for 12–13 weeks after cessation of treatment. The TWA doses were 590 and 1,179 mg/kg bw/day. Mice of both sexes showed a significant increase in the incidence of hepatocellular carcinoma.

The multistage model was used for high-to-low dose extrapolation (Crump and Watson, 1979; Howe and Crump, 1982), and the 95% upper confidence limit was used to assess risk as an upperbound estimate. The multistage model conforms to a biological model of tumor initiation and promotion (Crump et al., 1977). The relationship of the concentration (ug/L) of a chemical in drinking water to cancer risk is expressed as follows:

$$\frac{35{,}000 \times R}{q_1^*} - C$$

where:

q_1^* = $(mg/kg/day)^{-1}$

R = risk (10^{-4}, 10^{-5}, 10^{-6}, etc.)

C = concentration of chemical in ug/L

35,000 = conversion factor for mg to ug and assumption that a 70 kg adult drinks 2 L of water/day

The administered doses were TWA-adjusted for frequency of exposure (i.e., from 5 to 7 days). Human equivalent doses were adjusted for length of exposure (546 days of a potential lifespan of 637) and weight of the animals (assumed to be 0.032 kg). The vehicle control group incidence data were used in modeling.

The human slope factor (q_1^*) is 1.4×10^{-2} $(mg/kg/day)^{-1}$ for the linearized multistage model. The q_1^* is taken as an upper bound of potency of the chemical to induce cancer at low doses below the experimental dose range. Assuming that a 70 kg adult consumes 2 L of water a day over a 70-year lifespan, the estimated cancer risk is as follows (the dose is rounded):

Level of Human Risk	Dose in ug/L
10^{-4}	300
10^{-5}	30
10-6	3

For comparison purposes, drinking water concentrations associated with an excess cancer risk of 10^{-6} were 1 ug/L, 200 ug/L, 5,000 ug/L, 50 ug/L, and 2 ug/L for the one hit, multihit, probit, logit, and Weibull models, respectively. The parameters estimates for these models were calculated with RISK-81 (Kovar and Krewski, 1981).

The estimated excess lifetime cancer risk associated with lifetime exposure to drinking water containing hexachloroethane at 60 ug/L is approximately 2×10^{-5}. The estimated excess lifetime cancer risk associated with lifetime exposure to drinking water containing hexachloroethane at 6 ug/L is approximately 2×10^{-6}. This represents the 95% upper confidence limit on risk from extrapolation using the linearized multistage model. The actual risk is unlikely to exceed this value.

VIII. OTHER CRITERIA, GUIDANCE AND STANDARDS

The ACGIH (1986) 8-hour, time-weighted, average threshold limit value (TWA-TLV) for exposure to HCE is 10 ppm (≈100 mg/m^3). In 1990, ACGIH proposed to lower the TWA-TLV to 1 ppm (9.7 mg/m^3) and to classify HCE as an A2 carcinogen (suspect human carcinogen; based on limited evidence of carcinogenicity in experimental animals) (ACGIH, 1990; Hexachloroethane, 1990). The Occupational Safety and Health Administration permissible exposure limit (PEL) as indicated in NIOSH (1985) remains at 1 ppm (≈10 mg/m^3) for an 8-hour TWA. NIOSH (1985) recommends that HCE be treated as a carcinogen and exposure be limited to the lowest feasible limit.

The U.S. EPA (1980) published HCE ambient water quality criteria for human health at 19 ug/L, 1.9 ug/L, and 0.19 ug/L associated with lifetime excess cancer risks of 10^{-5}, 10^{-6}, and 10^{-7}, respectively. The criteria assumed that contaminated aquatic organisms are consumed in addition to water.

In Russia, the maximum allowable concentration (MAC) in reservoir water is 0.01 ppm (Sax, 1986), while the recommended maximum contaminant level (RMCL) is reported to be 10 g/L (Davidson et al., 1988).

IX. ANALYTICAL METHODS

Analysis for HCE in water generally involves an extraction step with an organic solvent followed by analysis using various modifications of gas chromatographic techniques.

In 1982, Otson and Williams described a modification of a purge and trap technique for the analysis of organics in water. In this procedure, a 40 mL aliquot water sample was injected into a modification of a Bellar and Lichtenberg glass purge assembly which was immersed into a 40°C temperature-controlled water bath for 20 minutes. Ultra high purity nitrogen was then bubbled through the device at a rate of 40 mL/min for 30 minutes and on-column trapping was initiated. The Tenax GC column (60/80 mesh), held at 40°C during the purging phase, was raised at a rate of 20°C/min to 100°C and then at 8°C/min to 190°C where it was maintained for 6.75 minutes. Nitrogen carrier gas, at a rate of 37 mL/min, was directed to either a flame ionization detector (FID) or a Hall electrolytic conductivity detector (HECD).

Hexachloroethane was detectable to 1 ug/L of water on the HECD detector with an electrolytic solvent of 2-propanol/water (50/50, v/v), hydrogen gas flow of 10 mL/min and a furnace temperature of 820°C. Retention time, relative to the start of the temperature program, was 14.9 minutes and resulted in its detection as a broad peak. A straight line was obtained when the concentration was plotted against peak area for aqueous standards over the range of 0.25 to 16 ug/L. Precision, as determined from triplicate analyses of aqueous standard samples at 16 ug/L, was reported as 12% relative standard deviation. Concentrations above 16 ug/L were not evaluated. Purging efficiency using the HECD, as percent recovery by comparison of peak area values from dynamic headspace analysis of aqueous standards at 40°C and direct injection analysis of equivalent methanolic standard solutions, was reported to be 82%.

A similar method for analysis of organics in water was described by Pankow et al. (1982) and involved the adsorption of the organics from aqueous solutions onto a polymeric sorbent followed by thermal desorption (ATD) with analysis by capillary gas chromatography (GC). The problem of desorption from a large volume cartridge [bed length 6.0 cm, inside diameter (i.d.) 0.69 cm] with a flow rate of approximately 30 mL/min to a fused silica capillary column (length 30 m, i.d. 0.25 mm) with a flow rate of approximately 1–2 mL/min was handled by addition of an interface cartridge between the two systems. To insure good chromatographic resolution without loss of sensitivity, the authors used a cold-trap method to retain the analytes at the head of the GC column until desorption was complete and GC analysis could proceed. A small, fixed 35/60 mesh Tenax GC interface cartridge (bed length 5.0 cm, i.d. 0.40 cm) made of Pyrex glass was connected between the Pyrex glass sample cartridge (packed with 35/60 mesh Tenax-GC) and the DB-5 (SE-54) fused silica capillary column by means of a short piece of small inside diameter (0.5 mm) glass-lined stainless steel tubing. During the desorption process, the column was maintained at a subambient temperature of -30°C with liquid nitrogen to trap the analytes at the column head. Analysis was carried out with a flame

ionization detector at 300°C with helium as the carrier gas. The column was heated ballistically from -30 to 50°C and then at a rate of 4°C/min from 50 to 250°C. Recovery efficiency for HCE by this method was reported to be between 62 and 75%. Detection limits for this procedure were not determined but were predicted to be in the 1.8–3.8 ng/L range.

Eichelberger et al. (1983) compared the scope and limitations of two methods of simultaneous identification and measurement processes for HCE and other organics in a complex sample matrix. In Method 625, the analytes were twice partitioned between pH adjusted water and methylene chloride in a separatory funnel, first at pH 11 and then at pH 2. Extracts were analyzed separately after drying and concentration to a low volume. An internal standard, anthracene-d_{10} was added to each extract. Analysis was by gas chromatography interfaced with mass spectrometry, with an all-glass transfer line and an all-glass, jet-type enrichment device. The column was a 1.8 m x 2 mm i.d. glass tube packed with 3% SP-2250 on 100/120 mesh Supelcoport. The injector temperature was 260°C and the transfer line temperature was 240°C with helium as the carrier gas at a flow rate of 30 mL/min. With this method, a 3.8 ug/L concentration of HCE in water had a mean recovery of 76% and a standard deviation of 23% of the mean recovery value.

Method 625.1 used essentially the same extraction procedure with the exception that the first separation was at pH 7 and the second remained at pH 2. Three internal standards, naphthalene-d_8, anthracene-d_{10} and chrysene-d_{12}, were added to the dried concentrated extract for GC analysis. In this method, the capillary column was of fused silica (28 m x 0.25 mm i.d.) coated with SE 54. The column temperature was maintained at 40°C for 4 minutes and programmed to increase at 10°C/min to a temperature of 275°C. The injector and interface oven temperatures were maintained at 260°C and the helium carrier gas had a linear velocity of 28 cm/sec. This method also interfaced with a mass spectrometer. Mean recovery was 55% for a 10 ug/L sample of HCE in water, with a standard deviation of 6.2% of the mean recovery value.

Fisher et al. (1985) used a modification of Standard Method 509A (APHA et al., 1985) to analyze chlorinated organics in water. Pentane (HPLC grade) extraction of a 50 mL water sample and concentration to 5 mL was followed by analysis using GC and an electron-capture detector. Quantification was by means of a Spectra-Physics integrator with standards prepared in the HPLC grade pentane. The extract was injected onto a column (6 ft x 4 mm i.d.) packed with 100/120 mesh Gas Crom Q support with an OV-210 stationary phase. Injector temperature was 100°C; carrier gas was nitrogen at 48 cc/min. The electron capture detector was set at 350°C and the oven was programmed for an initial one-minute hold at 80°C with an increase of 15°C/min to 160°C. Following a seven-minute hold, the oven was cooled to the initial 80°C.

A linear correlation was evident between peak area and concentration when levels were between 0.1 and 10 mg/L, with loss of linearity at higher levels. Retention time for HCE was 3.17 minutes and limits of detection were reported to be 0.01 mg/L for the concentrated pentane extracts of the 50 mL water samples.

X. TREATMENT TECHNOLOGIES

No information was found in the available literature on technologies useful for the removal of HCE from water. Studies of environmental fate indicate that HCE is acted upon by bacteria in pond waters under both aerobic and anaerobic conditions. Under anaerobic conditions, approximately 90% of the HCE content was lost in 18 days (Spanggord et al., 1985). Additional studies indicate that HCE is biotransformed to tetrachloroethylene in aerobic cultures under laboratory conditions. Transformation was constant with time in the presence of municipal sludge wastewater (Criddle et al., 1986). Volatilization is also a major transport process for HCE in water (Spanggord et a., 1985). It is likely that these environmental processes can be utilized to develop a method to remove quantities of HCE from water.

XI. CONCLUSIONS AND RECOMMENDATIONS

Based on the available animal data, the HA for One-day and Ten-day exposures for a child is 5 mg/L. The Longer-term HA for the child is 130 ug/L and for the adult is 450 ug/L. Evidence is presented that HCE may meet the EPA criteria for classification as C, Possible Human Carcinogen, based on animal data. The DWEL for HCE is 40 ug/L for lifetime exposure. The lifetime HA is 1 ug/L assuming a 20% RSC and equivocal evidence of carcinogenicity (Group C).

As indicated in the companion report, "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for Hexachloroethane" (Appendix 1), standard reproductive toxicity studies were not available and should be considered in future medical research plans.

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APPENDIX 1

Data Deficiencies/Problem Areas and Recommendations for Additional Database Development for Hexachloroethane

INTRODUCTION

The Office of Drinking Water (ODW), Environmental Protection Agency (EPA), in conjunction with the Department of the Army, has reviewed the available data on hexachloroethane (HCE) for the purpose of developing a Health Advisory (HA) useful in dealing with contamination of drinking water, to include "state-of-the-art" information on environmental fate, health effects and analytical methodology.

OBJECTIVES

The objective of this appendix is to provide an evaluation of the data deficiencies and/or problem areas encountered in the review process for HCE and to make recommendations, as appropriate, for additional database development. This document is presented as an independent analysis of the current status of HCE technology, as related to its possible presence in drinking water, and includes a summary of the background information used in the development of the HA. For greater detail on the toxicology of HCE the Health Advisory on Hexachloroethane should be consulted.

BACKGROUND

Hexachloroethane, a chlorinated alkane with a camphor-like odor, is used primarily by the military in pyrotechnic devices and screening smokes. It has more limited usage commercially in lubricants, insecticides, moth-repellents, fire-extinguishing fluids and submarine paints and as a solvent, chemical precursor and rodenticide. It is manufactured by the chlorination of tetrachloroethylene in the presence of ferric chloride at temperatures between 100 and 140°C (NIOSH, 1981). Most HCE is imported, with current U.S. production limited to its formation as a co-product in the manufacture of other chlorinated ethanes (Archer, 1979 as cited in Santodonato, 1985).

Smoke compositions contain HCE at levels averaging approximately 45% (Davidson et al., 1988). Once combusted, several by-product gases are formed with HCE amounting to approximately 0.3 to 5% of these gases (Katz et al., 1980). Zinc chloride is the major metallic by-product (Novak et al., 1987). Its presence in the environment is largely due to its military and industrial uses, as well as from the load and pack procedures for the smoke devices. It is not found as such in nature. It has been measured in surface waters at 1–3 ug/L (Ewing et al., 1977 as cited in Davidson et al., 1988), in drinking water at levels of 0.03–4.3 ug/L (Keith et al., 1976 as cited in IARC, 1979), and in air at 0.3–49 ug/m^3 (Silkworth et al., 1984 as cited in Davidson et al., 1988). Once in water, volatilization and biotransformation have been shown to occur. Volatilization half-lives between 40 minutes and 70 hours have been estimated (Dilling, 1977 as cited in Callahan et al., 1979; Spanggord et al., 1985) while a biotransformation half-life of approximately 40 days was determined in an unconfined sand aquifer (Criddle et al., 1986). When released to the atmosphere, HCE is relatively stable (Davidson et al., 1988).

The pharmacokinetic properties of HCE have been studied in sheep, rabbits, rats and mice. Absorption has been demonstrated after oral, dermal and inhalation exposure, with distribution largely to fat. Concentration in the kidneys of male rats has also been demonstrated (Gorzinski et al., 1985). Excretion is mainly via the expired air with 65 to 71% of the administered dose recovered following multiple oral doses in rats and mice (Mitoma et al., 1985). Recovery in combined excreta ranged between 6 and 16%. Tetrachloroethylene is the major metabolite in sheep (Fowler, 1969) while trichloroethanol and trichloroacetic acid were the major metabolites in mice and rats (Mitoma et al., 1985). In an in vitro study with rat liver microsomes, tetrachloroethene was the major metabolite (Nastainczyk et al., 1982).

Data on the exposure of humans to HCE are very limited, with reports of mild neurological effects as well as direct effects on the eye following acute exposure (Grant, 1986). Irritation was the only effect reported after chronic exposure in the workplace (Dacre, 1979).

Oral LD_{50}s in rats range between 5,160 and 7,690 mg/kg in males and 4,460 to 7,080 mg/kg in females, depending upon the vehicle used. The oral LD_{50} in male guinea pigs was reported at 4,970 (Weeks et al., 1979; Weeks and Thomasino, 1976). Toxic signs include tremor, ataxia, signs of gasping, and the appearance of a red exudate around the eyes. Oral doses up to 1 g/kg in sheep resulted in signs of liver toxicity as demonstrated by an increase in plasma hepatic enzymes and a marked reduction in transfer from liver to bile in the BSP clearance test (Fowler, 1969). No effects were demonstrated on the chemical or functional properties of liver microsomes of rats that were orally dosed with HCE at a level equivalent to approximately 6 g/kg (Reynolds, 1972).

Weeks et al. (1979) reported the development of a significant decrease in body weight gain, increased relative liver and kidney weights, and liver and kidney histopathology in rabbits orally administered HCE at doses up to 1,000 mg/kg/day for 12 days. A NOAEL of 100 mg/kg/day was determined. In a 16-day study in rats, dietary intake of HCE at doses up to 500 mg/kg/day also produced a significant decrease in body weight gain, increased kidney weight in males as well as gross liver and kidney lesions (Gorzinski et al., 1980a). A NOAEL of 50 mg/kg/day was determined.

In a six-week screening study designed to determine the maximum tolerated dose (NCI, 1978), toxicity was demonstrated at levels ≥562 mg/kg/day in rats, and was evidenced by weight loss and mortality. In mice, toxicity was demonstrated at doses ≥1,780 mg/kg/day. Doses in this study ranged between 178–1,780 mg/kg/day in rats and 316–3,160 mg/kg/day in mice.

Skin and eye irritation studies indicated that HCE is a mild skin irritant in rabbits (Weeks and Thomasino, 1976), while moderate corneal opacity, iritis, swelling and discharge occurred upon exposure of the eye to direct contact with the dry technical HCE (Weeks et al., 1979). No skin sensitization was evidenced in guinea pigs (Weeks and Thomasino, 1976). A dermal LD_{50} of ≥32 g/kg was also reported.

Inhalation studies were conducted in dogs, rats, guinea pigs and quail over a period of 6 weeks at doses up to 260 ppm (approximately 2,515 mg/m^3). Exposure was for 6 hours/day, 5 days/week (Weeks et al., 1979) The only toxic sign was the appearance of a mucopurulent exudate in rats and quail, and mild CNS effects in dogs. When HCE was administered by inhalation on Days 6–16 of gestation, maternal toxicity in rats was demonstrated by a significant decrease in weight gain at both 48 and 260 ppm. No anomalies were detected in the fetuses and no other changes occurred in maternal and fetal parameters.

Gorzinski et al. (1980b) reported toxic effects on the liver and kidneys, demonstrated by histopathological changes, in rats administered HCE in the diet at levels up to 100 mg/kg/day for 16 weeks. Kidney lesions were characterized by a renal tubular atrophy and degeneration in the male rats at doses of 30 and 100 mg/kg/day, while in females at the high dose, kidney lesions were very slight. Liver lesions were characterized by a swelling of the hepatocytes, and were observed in the high-dose males only. No effects were seen on body weight, food intake and most clinical chemistry parameters evaluated. Actual intake levels for this study were analyzed at 1.3, 20 and 82 mg/kg/day. A NOAEL of 1.3 mg/kg/day was indicated.

In a bioassay for carcinogenicity (NCI, 1978), rats and mice were exposed to HCE by oral intubation at doses of 212 and 423 mg/kg/day in rats and 590 and 1,179 mg/kg/day in mice for 78 weeks of exposure. Kidney lesions were seen in both species at both the low and high doses. An increase in the incidence of interstitial cell tumors of the testes was reported in the high-dose rats. No liver lesions were reported in the rat, while a significant increase in hepatocellular carcinomas was reported in both sexes of mice at both dose levels.

In a 2-year gavage study of HCE in Fischer-344 rats, an increased incidence of hyperplasia, adenomas and carcinomas of the kidneys was seen in the males but not the females (NTP, 1989). The incidence of nephropathy, however, was increased in the females, although the severity of the lesions was mild. The males in this study were given doses of 10 or 20 mg/kg while the females were given doses of 80 and 160 mg/kg.

No information on the effects of HCE on reproduction in animals was found in the available literature. Developmental effects were studied in rats orally administered HCE on Days 6–16 of gestation at doses up to 500 mg/kg/day (Weeks et al., 1979). Hexachloroethane was not considered teratogenic, although signs of maternal and fetotoxic effects were indicated. Maternal rats displayed a significant decrease in weight gain and significantly lower gestations indices were reported. These included a decrease in the number of viable fetuses and an increase in fetal resorption at the high dose. A NOAEL for maternal and fetotoxic effects was 100 mg/kg/day.

Several analytical methods involving extraction with various GLC procedures have been described (Otson and Williams, 1982; Pankow et al., 1982; Eichelberger et al., 1983; Fisher et al., 1985). No treatment technologies were found in the available literature.

Based on the foregoing studies, HA values for One-day and Ten-day exposures were recommended at 5 mg/L. The Longer-term HA for the 10 kg child is 100 ug/L and for the 70 kg adult, 450 ug/L. The Lifetime HA is 1 ug/L assuming a 20% RSC and equivocal evidence of carcinogenicity (Group C). A DWEL of 40 ug/L was determined.

DISCUSSION

Available data on the pharmacokinetics, health effects and analysis of HCE have been reviewed.

The pharmacokinetic properties have been studied in various species and results indicate that HCE is absorbed following all exposure routes, is distributed largely to fat with kidneys of male rats also containing significant amounts. Excretion occurs mainly via the expired air with lesser amounts in the combined excreta. Major metabolites have been variously described. No data gaps are apparent; no additional studies are required.

Available studies on the health effects of HCE include oral LD_{50}s in rats and guinea pigs. A dermal LD_{50} has also been reported. Twelve- and 16-day toxicity studies indicate that HCE exerts some toxic effects on the liver and kidney over the short exposure duration, as well as causing significant decreases in weight gain in rats and rabbits. Similar effects on the kidney and liver were seen at lower doses in the diet of rats over a 16-week period, although body weight was not affected in this study. Similar findings on the kidney and liver were also evident after a lifetime exposure in rats and mice. Male rats developed a significant increase in interstitial cell tumors of the testes after a lifetime oral exposure to HCE. While this lifetime study did not establish a NOAEL or LOAEL for noncarcinogenic effects of HCE, the available data appears adequate for developing a Longer-term HA. No further studies are recommended at this time.

No studies were found on the reproductive effects of oral exposure to HCE in animals. Developmental toxicity studies indicated that HCE is not teratogenic in rats but significant maternal and fetotoxic effects were demonstrated. In view of the occurrence of interstitial cell tumors in the testes of rats, as well as the apparent fetotoxic effects, it is recommended that standard reproduction studies be conducted to evaluate this parameter.

A 78-week bioassay study in mice and a 2-year bioassay in rats indicates that HCE may be classified as a carcinogen, based on the development of a significant incidence of hepatocellular carcinomas in mice and renal adenomas and carcinomas in rats after oral exposure to HCE. Further evaluation of these studies, as well as of the 78-week bioassay in rats, is suggested before recommendations for additional studies are made.

No evidence of mutagenicity was seen in several assays for genotoxicity. No further assays are recommended at this time.

Several methods for the analysis of HCE are available. Limits of detection indicate that they are adequate for the detection of HCE in water at levels currently considered to be harmful to man. No further studies are required.

No treatment technologies for the removal of HCE could be found in the literature. It is recommended that studies be undertaken to develop such methods in the event that HCE should enter the water supply at levels above those currently found.

CONCLUSION/RECOMMENDATIONS

Based on the above discussion, the following conclusions/recommendations can be made:

1. The available studies on the toxicity of HCE are generally considered adequate for development of a HA useful in dealing with potential contamination of drinking water.

2. In view of the toxic effects of HCE to the testes of male rats, and considering its apparent maternal and fetotoxic effects, it is recommended that a standard three-generation reproduction study be undertaken to evaluate the effects of HCE on this parameter.

3. It is recommended that methods be developed for the removal of HCE from drinking water in the event that a high level contamination occurs.

4. Aside from the aforementioned data gaps, no other studies on HCE, as related to its possible presence in drinking water, are recommended at this time.

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